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# BULLETIN

OF

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## STUDIES OF DIABETES MELLITUS

### III. THE USE OF THE PANCREATIC EXTRACT INSULIN IN THE TREATMENT OF DIABETES MELLITUS

By Wm. S. McCANN, M. D., R. ROGER HANNON, M. D., and KATHERINE DODD, M. D.

(From the Departments of Medicine and Pediatrics of the Johns Hopkins Hospital)

Early in August 1922, through the kindness of Dr. G. H. A. Clowes of the Eli Lilly Research Laboratories, it was possible to commence the study of the effects of Iletin (Insulin, Lilly), in the treatment of diabetes mellitus in the clinics of the Johns Hopkins Hospital. For a preliminary report ten cases have been selected from about 40 cases studied in the Medical Clinic and in the Harriet Lane Home for Invalid Children. These cases have been chosen to illustrate the use of this remarkable therapeutic agent in its present state of development. They include both successes and failures. At the present time only the immediate effects of treatment can be discussed. Not until considerable time has elapsed will it be possible to evaluate the new drug completely in regard to its effects upon the duration of life and on the

incidence and control of complications in patients with diabetes mellitus.

Although little more than a year has passed since Banting and Best<sup>1</sup> made their epoch-making preliminary report of the discovery of an active extract of the pancreas, the literature has become quite voluminous. A complete summary of the publications on insulin up to August 1922 has been made by Allen.<sup>2</sup> Up to the time of this writing the publications have dealt principally with physiological studies of insulin, and with methods of preparation and assay.

<sup>1</sup> Banting, F. G., and Best, C. H.: The internal secretion of the pancreas. *Jour. Lab. Clin. Med.*, 1922, VII, 251-256.

<sup>2</sup> Allen, F. M.: *Jour. Metab. Research*, 1922, II, 125.

The methods of study of the cases presented here have been described in detail in paper II of this series.<sup>3</sup> In Table III, Case 9, a portion of the tabular record kept on each case history has been reproduced. Since many of these records are quite long it was felt that, for clearness and brevity, it was better to present only a graphic record of the data. In the figures which accompany the case abstracts there is no record of the diets given to the patients. The columns at the bottom of each chart represent the weights in grams of the foodstuffs actually metabolized by the patients. Solid black columns represent the weight of carbohydrate utilized, which is taken to be the difference between carbohydrate ingested and that excreted in the urine. Columns with diagonal shading represent the amount of protein metabolized as determined from the amount of urine nitrogen excreted per diem (Urine N. gm.  $\times$  6.25). Knowing the amounts of protein and carbohydrate oxidized per diem, one needs only to know the amount of heat produced in 24 hours in order to calculate the amount of fat burned. It is obvious that the heat, which is not supplied by proteins and carbohydrates, must have been derived from the oxidation of fats.

If patients are kept in bed, a fairly accurate estimate of the heat production per diem may be made by adding 10% to the basal metabolism. This addition allows for the specific dynamic effect of food and for the slight activity of life in bed. The basal metabolism was determined by measurement of the respiratory exchange. In order to estimate the amount of fat oxidized per diem the sum of the calories derived from protein and carbohydrate was subtracted from the total heat production; the difference divided by 9.3 was the amount in grams of fat burned.

Basal heat production plus 10% = total calories per diem.

Total Cal.—4.1 (Prot. + Carb.) gm.

$$\text{Total Cal.} - 4.1 \quad (\text{Prot.} + \text{Carb.}) \text{ gm.} \\ = \text{Fat gm.} \\ 9.3$$

In certain cases, in estimating the total calories, allowance has been made for the presence of fever and for heat lost in ketone bodies excreted. Usually patients were allowed to sit up and to walk only after glycosuria and ketonuria had ceased for several days. When this extra activity was allowed it was assumed that all of the fat of the diet was burned.

In making up diets the ketogenic-antiketogenic balance was maintained by employing the formula of Woodyatt,<sup>4</sup> that is,  $F=2C+P/2$ . When this is true, the ratio of total available fatty acids to total available glucose in

<sup>3</sup> McCann, Wm. S., Hannon, R. R., Perlzweig, W. A., and Tompkins, E. H.: Studies of Diabetes Mellitus. II. Results of treatment by diet adjustment with reference to maintenance requirement and the ketogenic-antiketogenic balance. Arch. Int. Med., in press (July 1923).

<sup>4</sup> Woodyatt, R. T.: Object and methods of diet adjustment in diabetes mellitus. Arch. Int. Med., 1921, XXVIII, 125.

grams is as 1.5 : 1, and the molecular ratio of ketogens to antiketogens is as 1 : 1. The method of Woodyatt has been modified in that the initial diets given in this clinic were very low in protein. A graphic method has been described<sup>5</sup> for adapting Woodyatt's formula in making a diet prescription for any desired number of calories and with any desired proportion of protein calories to the total heat value of the diet. The application of the method is given in detail in paper II of this series.<sup>3</sup>

The dosage of insulin has been given in units as determined by the manufacturer's assay. A unit has been defined<sup>6</sup> as the amount of insulin per kilogram which causes the blood sugar of rabbits to decrease to 0.045% within 4 hours.

#### ABSTRACTS OF CASE HISTORIES

CASE I. Donahue, a man aged 31 years, admitted July 19, 1922, complaining of thirst, weakness, numbness and pins and needles sensations in the feet, and boils. Duration, sixteen months.

Onset in March 1921. Sugar was discovered in the urine at that time. The diet was restricted until July 1921, after which it was not adhered to. Numbness and "pins and needles" sensation increased. Boils were frequent. There was occasional diplopia. For six months before admission there was loss of libido and potentia sexualis. There was a loss of weight of forty-eight pounds (135-97) up to the time of admission.

P. H.:—Venereal history negative, but his wife had had six miscarriages following the birth of one healthy child now six years old.

F. H.:—Mother died of tuberculosis.

*Physical Examination* (positive findings: Marked emaciation; no odor of acetone; skin, dry and harsh with xanthomata diabetorum over the extensor surfaces of arms and legs, especially about the elbows and knees, a few being in the flexor surface. Heart and lungs normal. B. P. 90/60. Deep reflexes not obtained, except the triceps reflex of the left arm.)

*Urine Examination*:—Pale, frothy, sp. gr. 1028, contained albumin, sugar, acetone, diacetic acid.

*Blood*: Wassermann reaction, negative.

*Blood sugar*: 0.210%.  $\text{CO}_2$  combining power of plasma: 43.9%.

*X-ray report*: Chest—lungs clear.

The diets given from July 20th-October 16th are shown in Table I.

The course of the patient under treatment is shown graphically in Figs. 1 and 2. The point of greatest interest lies in the fact that, after the diabetes had been controlled for a period with insulin, it was possible to discontinue the use of insulin for about three weeks and simultaneously to increase his diet enormously. When glycosuria again occurred on October 17th, the carbohydrate tolerance again decreased. It was necessary to use larger doses of insulin in order to render the urine sugar-free once more. The patient was discharged with a diet prescription as follows: protein 40 gm., fat 150 gm., carbohydrate 65 gm. During a trial of life on this diet for one

<sup>5</sup> Hannon and McCann: A graphic method for the calculation of diabetic diets in the proper ketogenic-antiketogenic ration. Johns Hopkins Hospital Bulletin, 1922, XXXIII, 128.

<sup>6</sup> Banting, Best, Collip, McLeod, and Noble: The effect of the pancreatic extract insulin on normal rabbits. Am. Jour. Physiol., 1922, LXII, 162.

week after leaving the hospital he remained in good condition, urine sugar-free and blood sugar normal. It was not possible to continue insulin treatment. The subsequent course is unknown.

In Fig. 3 one may see the results of respiration experiments carried out on this patient. On September 12th there is shown a marked depression of respiratory quotients when glucose was ingested following elevation of the respiratory quotient by insulin injection. A week later the depressing effect of glucose on the R. Q. after insulin was still present but less marked when the experiment was repeated. A respiratory glucose tolerance test made six days later showed no depression of respiratory quotient following glucose ingestion.

TABLE I.

Dates	DIET			
	Protein gm.	Fat gm.	Carbo. gm.	Cals.
July 20-23 . . . . .	50	75	40	1070
July 24 . . . . .	35	115	48	1375
July 25-31 . . . . .	20	90	40	1180
Aug. 1-2 . . . . .	13	2	38	228
Aug. 3-10 . . . . .	20	90	40	1180
Aug. 11-17 . . . . .	35	110	40	1330
Aug. 18 . . . . .	13	2	38	228
Aug. 19-Sept. 2 . . . . .	20	90	40	1180
Sept. 3-15 . . . . .	20	150	70	1770
Sept. 16-30 . . . . .	35	150	70	1830
Oct. 1-4 . . . . .	35	150	85	1890
Oct. 5-7 . . . . .	35	150	105	1975
Oct. 8-11 . . . . .	35	150	125	2055
Oct. 12-13 . . . . .	35	150	145	2140
Oct. 14 . . . . .	35	150	155	2180
Oct. 15 . . . . .	35	150	175	2260
Oct. 16 . . . . .	35	150	195	2340

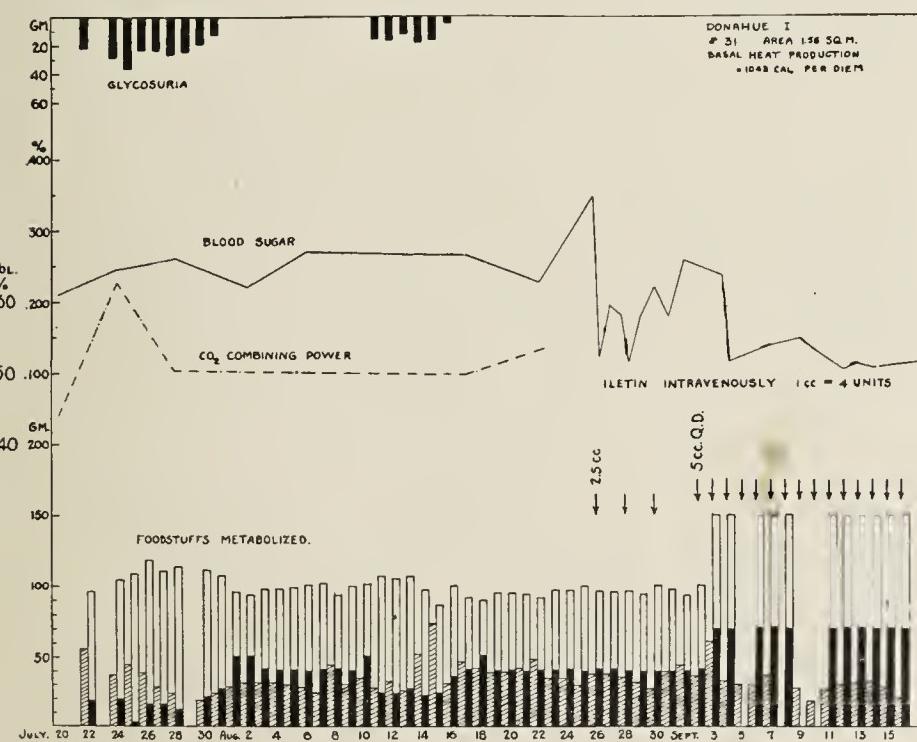


Fig. 1. Case 1. Black columns represent carbohydrate in grams. Diagonally shaded columns represent protein metabolized; unshaded columns the fat. Levels of blood sugar before breakfast are shown.

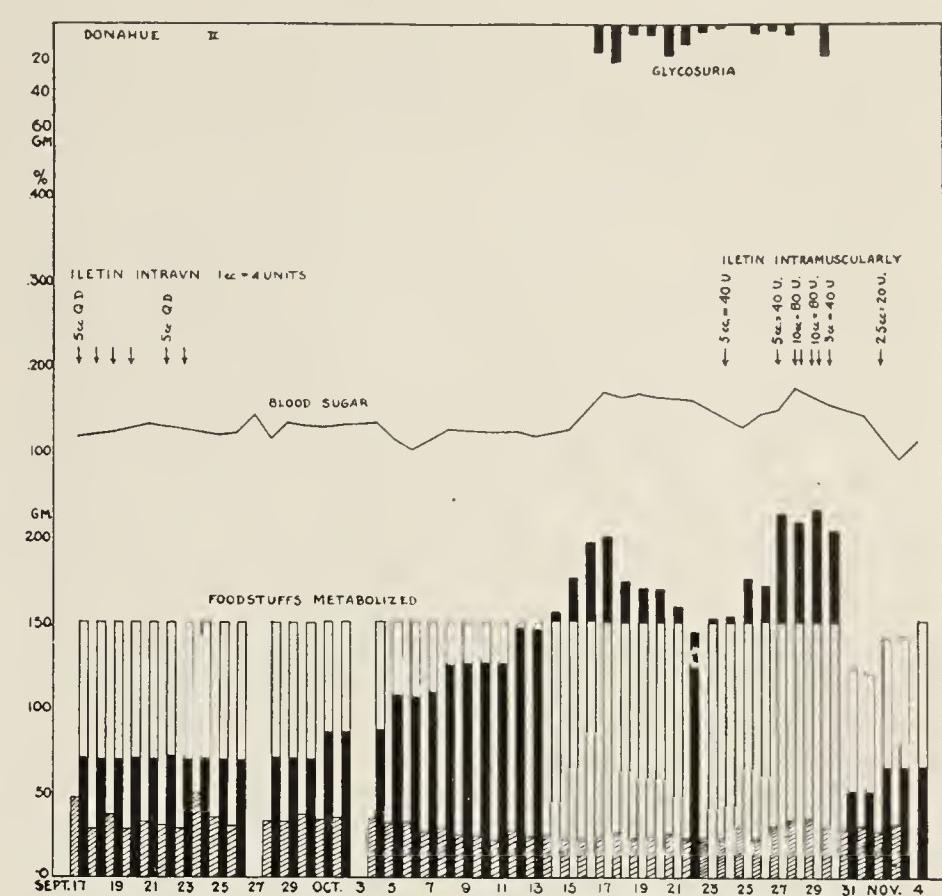


Fig. 2. Case 1. Continuation of record from Fig. 1.

These observations are interpreted as follows: The drop in quotient may be explained by the tentative assumption that glycogen stores, being greatly depleted, tend to be filled when sugar is given after insulin injection. This behavior is similar to that seen in severe diabetes when insulin has not been given. The greater the depletion of glycogen reservoirs the more marked is the drop in quotients. The successive experiments may be taken as showing improved ability to oxidize sugar, due to a gradual building up of the glycogen stores by the treatment with insulin.

CASE 2. Adele H., aged 6½ years when admitted first to Harriet Lane Home in March 1921, because she was thin and

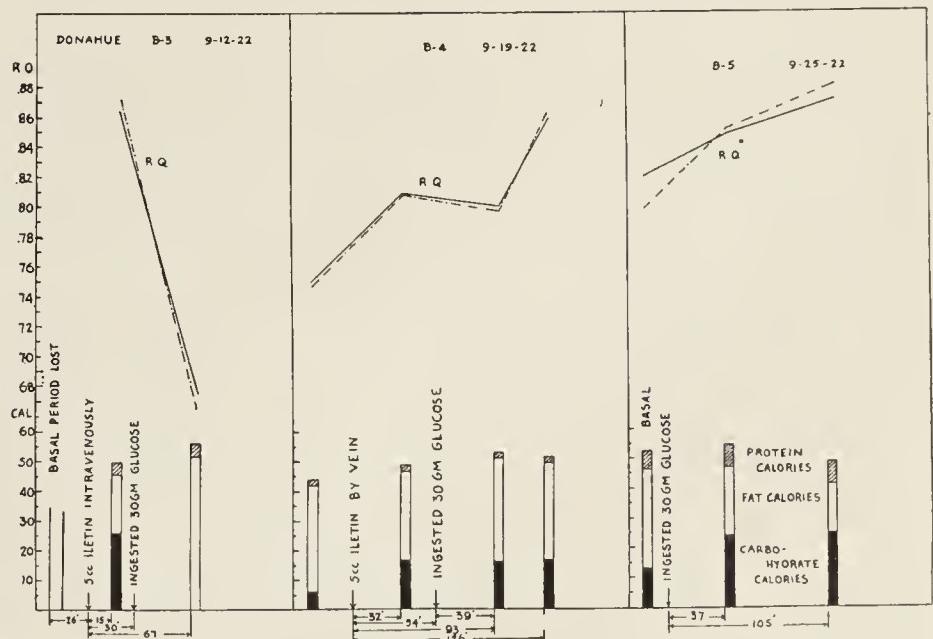


Fig. 3. Case 1. Data of respiration experiments. From the R. Q. and urine N. excretion the amounts of food stuffs metabolized were calculated. Black represents calories from carbohydrate, white calories from fat, and diagonally shaded portion calories from protein. The height of the column represents the heat production.

tired. Family history negative except that paternal grandparents had tuberculosis. Past history—birth three weeks premature. Infections: pneumonia at 9 months, mumps at 3½ years, scarlet fever at 5 years, whooping cough at 6 years. Development: normal.

*Present Illness:* One and one-half years before admission, following scarlet fever, the mother noticed that the patient seemed continually tired, and that she was getting thinner. She seemed nervous and restless at night. She began to drink more water and polyuria was noticed. Slight transient rashes were noted on her body from time to time, associated with itching. Two and a half months before admission patient developed pertussis. The urine was said to have been examined in December 1920, but no sugar was reported. A second urine examination in February 1921 showed an abundance of sugar (4½%).

*Physical Examination:* March 4, 1921. Age 6½ years. Temperature, pulse and respirations normal. Weight 44 pounds. Moderately well nourished. Mentally active. Small urticarial wheals were noted over lower part of trunk. Further examination negative, except for soft systolic murmur at apex of heart. Leucocyte count 12,200. Urine of high specific gravity, 1034, contained much sugar and a trace of albumin, otherwise negative. There was no acetonuria.

*Course:* In hospital twenty-eight days, during which she lost three pounds. Her tolerance for sugar was 130 gm. carbohydrate. Diet on discharge: protein 75 gm., fat 80 gm., and carbohydrate 70 gm., giving 69 calories per kilogram.

*Second admission,* July 7, 1921. Patient was in hospital one week. Carbohydrate tolerance 120 grams. Diet on discharge: protein 50 gm., fat 100 gm., and carbohydrate 60 gm., giving 69 calories per kilogram.

*Third admission,* October 9, 1921. In hospital sixteen days. Diet on discharge: protein 50 gm., fat 100 gm., carbohydrate 60 gm. Calories per kilogram 69. Weight 44 pounds. Tuberulin test negative. Schick test negative.

*Fourth admission,* February 5, 1922. In hospital five days. Physical examination: negative. Diet on discharge: protein 50 gm., fat 100 gm., carbohydrate 60 gm.

*Fifth admission,* May 3, 1922. In hospital three days. Diet on discharge: protein 50 gm., fat 110 gm., carbohydrate 40 gm.

August 29, 1922. Report of glycosuria on this diet about twice a week. An attempt was made to substitute glycerol for carbohydrate, but this was abandoned because it apparently caused urticaria. During September 1922 glycosuria occurred almost every other day. The diet was gradually reduced to protein 50 gm., fat 80 gm., carbohydrate 25 gm. The child became less lively and lost weight.

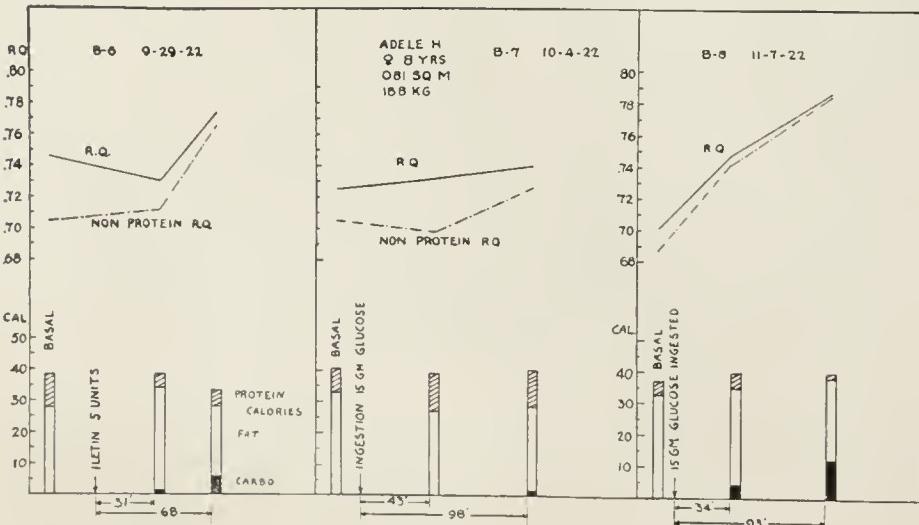


Fig. 4. Case 2. Data of respiration experiments. Symbols as in Fig. 3.

*Sixth admission,* September 24, 1922. Age 7 years, 11 months. *Physical examination:* negative, except for marked emaciation, and enlargement of several cervical lymph nodes. Blood sugar 0.288%. CO<sub>2</sub> combining power of plasma: 34 volumes per cent. The patient was given a diet as follows: protein 50 gm., fat 110 gm., carbohydrate 30 gm. Calories 1200. It was decided to use "Iletin" (Insulin, Lilly), but the early attempts at this treatment were unsuccessful partly through incorrect administration and partly because of low potency.

September 29. A meagre effect of insulin on the metabolism and respiratory quotients was noted after intravenous injection of five units. (See Fig. 4.)

Between September 30th and October 18, 1922, daily intravenous doses of five to ten units failed to produce appreciable success. Diet: protein 38 gm., fat 85 gm., carbohydrate 30 gm. Calories 1070.

October 19th and 20th. More potent insulin was obtained. Twenty units intramuscularly produced a disappearance of sugar from the urine in two hours, lasting for thirteen hours.

October 21st-25th. Intramuscular injections. Twenty units at 10 a. m. and 10 p. m. rendered the patient almost sugar-free. Diet: protein 38 gm., fat 85 gm., carbohydrate 30 gm. Calories 1070 per diem.

October 26th-28th. Iletin, intramuscular injection, thirty units at 9 a. m. and 8 p. m. Urine sugar-free. Diet unchanged. 1070 calories. Total units 60.

October 29th. Iletin sprayed in nose every half hour. Total units 30. Urine volume 3060 c.c. Contained 32.8 gm. sugar.

November 1st-4th. Injections b. i. d. 24 units. Total 48. Diet unchanged. Patient exhibited severe hypoglycemic reactions, in one of which a stupor occurred lasting several hours, with spontaneous recovery.

November 5th-6th. Injections b. i. d. 16 units. Total 32. Diet increased to protein 43, fat 100, carbohydrate 35 gm. Calories 1250. The patient still showed severe reactions. In the afternoon of November 6th, great pallor, irritability and abnormal mental symptoms developed. The blood sugar was 0.031%. Coma did not occur, as patient was given carbohydrate.

November 7th. Respiratory carbohydrate tolerance test: when 15 gm. glucose were ingested a normal curve of respiratory quotients was observed. (See Fig. 4.)

November 8th-11th. Injections: 12 units, b. i. d., total 24. Diet: protein 50 gm., fat 120 gm., carbohydrate 40. Total calories 1490.

November 12th-16th. Same diet. Dose reduced to 18 units in all, given in two injections at 8.20 a. m. and 4.30 p. m.

A great change occurred in the vivacity and strength of the child. Up to this time there had been no gain in weight.

#### SUMMARY

Date	Food Cals.	Urine sugar	Dose units	
Sept. 30-Oct. 18..	1070	+	10	Wt. 40 lbs.
Oct. 19-20 .....	1070	+	40	
Oct. 21-28 .....	1070	0	60	
Nov. 1-4 .....	1070	0	48	hypoglycemia
Nov. 5-6 .....	1250	0	32	hypoglycemia
Nov. 8-11 .....	1490	0	24	
Nov. 12-16 .....	1490	0	18	
Jan. 17 .....	1800	+	18	
Jan. 29 .....	1800	0	25-35	P. 68 F. 150 C. 56 } Wt. 56 lbs.

The remarkable change in her ability to oxidize glucose is shown graphically in Fig. 4, which gives the changes in the curves of respiratory quotients following the ingestion of a test meal of fifteen grams of commercial glucose. The experiment of November 7th shows a normal rate of increase in quotients after the ingestion of glucose.

*Subsequent Course:* After leaving the hospital the patient gained twenty pounds in weight and led a normal active life of an eight-year-old child. Her diet furnished 1800 calories, protein 68, fat 151, carbohydrate 56 grams. The dose of insulin required to maintain the urine sugar-free on this diet varied between 25-35 units per diem.

From the above data one may calculate the number of extra food calories which the patient could obtain per unit of insulin. Since only 1490 calories could be taken with the dose of 18 units and an addition of 7 units was required when the diet was increased to the value of 1800 calories, one may conclude that one unit of insulin enabled the patient to utilize extra food to the value of 44 calories.

It is much more difficult to arrive at any means of determining just how much the power to oxidize carbohydrate is increased per unit of the drug, especially in this case in which the nitrogen metabolism of the patient was not measured. With the data available such a calculation does not seem to be possible.

A more detailed description of the reaction following an overdose of insulin on November 4, 1922, is given by Dr. Dodd, who observed the occurrence.

The child was sitting up in bed talking and laughing. Suddenly, at 7 p. m. she became pale and slipped down to a recumbent position. Her eyes became wide and staring. The pupils were dilated. When her mother or doctor spoke to her she did not answer, but stared at them without recognition. She dozed part of the time but roused occasionally and smiled broadly and sometimes laughed a little. She voided in the bed. Her pulse remained good, but her hands and feet and forehead were cold and clammy. She did not sweat and had no convulsive movements. This condition continued with periods of dozing alternating with periods of wild looking around the room and foolish grinning until 10 p. m., when she smiled at her nurse and recognized her. Her blood sugar was not determined at that time, but on the next night, when she had a similar though less severe reaction following 2/3 of the dose given on Nov. 4, the blood sugar was found to be as low as 0.031%.

**CASE 3.** Wright, an oil field worker, aged 36 years, admitted first in July 1921, complaining of polyuria and thirst. Onset of symptoms in February 1918, at which time he was given treatment by starvation for ten days. He improved markedly, returned home, and continued to do well until April 1921. At this time he had a severe fall. Following this he had increasing difficulty in getting his urine sugar-free by fasting. He lost twenty to thirty pounds in weight.

*Family history:* His mother and one brother have goitres.

*Past history:* Unimportant.

*Physical Examination (positive findings):* Poor nutrition. Eyes normal. Tonsils large. Lymph nodes: epitrochlear and inguinal palpable. Heart normal. Lungs: slight impairment of percussion over right upper lobe, with a few dry râles heard at right apex. Reflexes normal. The urine showed sugar, no acetone. Phenolsulphonephthalein excretion 83% in two hours.

*Blood:* Wassermann reaction negative. R. B. C. 4,600,000; W. B. C. 6,700. Haemoglobin 82%. On admission the CO<sub>2</sub> combining power of the plasma was 55 vols. per cent., and the blood sugar was low, 0.08%. All subsequent chemical findings of blood on first admission were normal.

The patient was discharged in August 1921, with a diet containing 60 grams of carbohydrate, protein and fat not specified. Two weeks after discharge glycosuria recurred on this diet. He regulated this himself by reducing carbohydrate ingestion, and managed to do light work and to keep sugar-free most of the time for four to five months, during which he gained four to five pounds.

On July 4, 1922, the patient had an attack of nausea and vomiting with a fever (102° F.) lasting two days. Between July and October there were two or three similar attacks, with nausea, vomiting, abdominal discomfort, and shortness of breath. On October 26, 1922, he had another attack which was the immediate reason for his second admission.

*Physical Examination, October 27th:* Height 177.0 cm. Weight 55 kg. Findings as on first admission except for evidence of acetone on breath, hyperpnoea, and absent tendon reflexes. The prostate was somewhat tender, but not enlarged. Blood pressure 110/80. Urine: showed considerable sugar and acetone. Phenolsulphonephthalein excretion 52% in two hours. Blood: R. B. C. 4,660,000; W. B. C. 3,200.

#### Basal metabolism:

21% below average normal (DuBois and Aub).  
Calories per diem..... 1300  
plus 10% ..... 130

Calorie requirement ..... 1430

The patient's course under treatment is shown graphically in Fig. 5. For the sake of brevity the diet and metabolism table has been omitted.

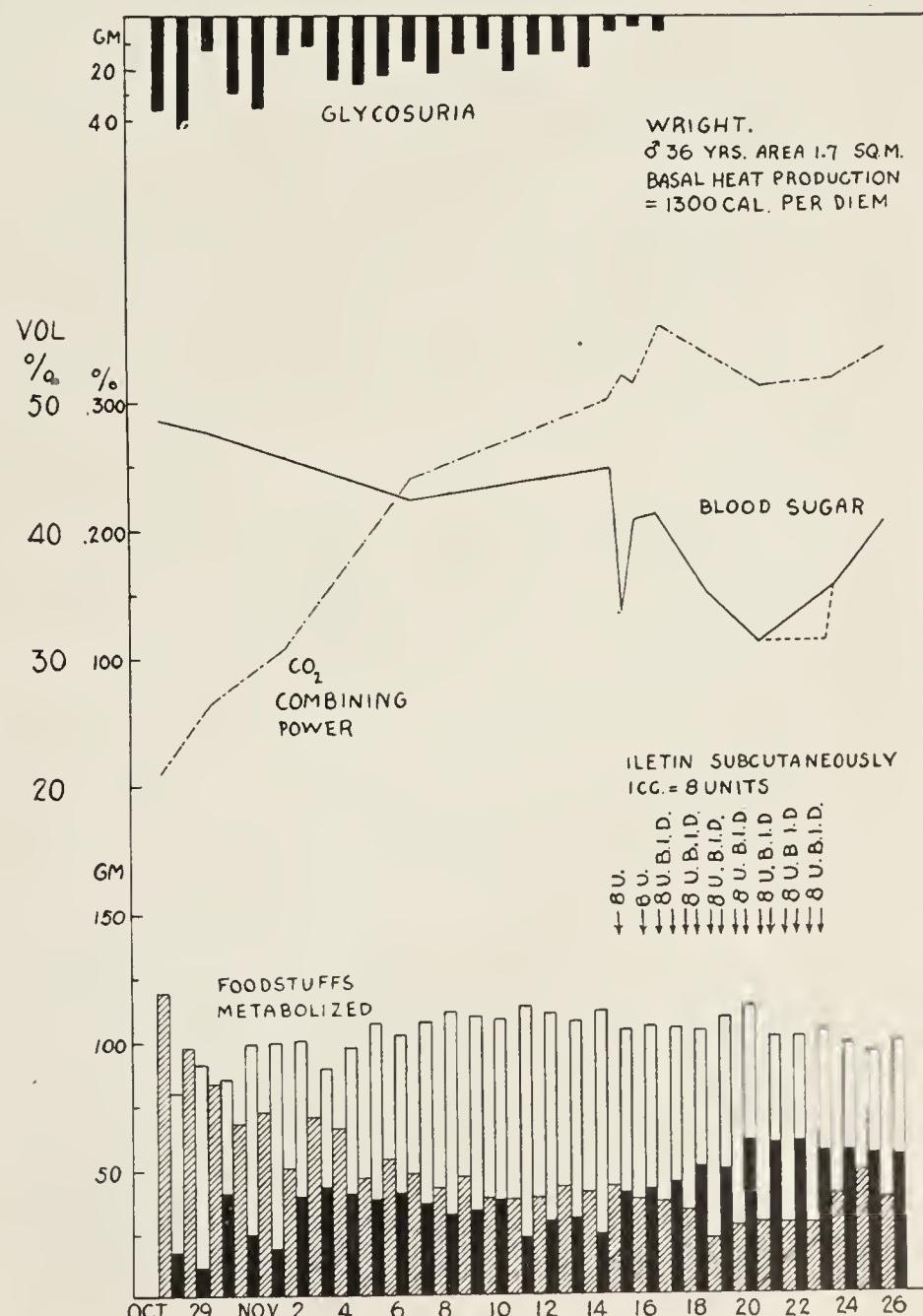


Fig. 5. Case 3. Symbols as in Fig. 1.

October 29th-November 7th. Diet, 1580 calories; protein 38 gm., fat 130 gm., carbohydrate 55 gm. During this period there was a rapid clearing up of the acidosis as shown by the rise in the curve of CO<sub>2</sub> combining power. Hyperglycemia and glycosuria persisted. The diet was in excess of the basal requirement.

November 7th-16th. Diet, 1350 calories; protein 32 gm., fat 116 gm., carbohydrate 45 gm. The rise in CO<sub>2</sub> combining power of blood plasma continued. Glycosuria diminished. Hyperglycemia persisted.

November 15th-16th. On each of these days 8 units of insulin (Iletin) were given. These produced transient effects, but glycosuria continued.

November 17th-19th. Diet increased and dose of insulin doubled. Diet, 1550 calories; protein 32 gm., fat 133 gm., carbohydrate 50 gm. Insulin 8 units b. i. d. Total 16 units. Glycosuria ceased. Blood sugar percentage fell to normal limits. On November 18th acetonuria ceased, FA/G ratio=1.6.

November 20th-22nd. Diet, 1870 calories; protein 32 gm., fat 165 gm., carbohydrate 60 gm. An increase of 520 calories in the diet was made possible by 16 units of insulin. Calories per unit=34.

November 22nd-24th. Diet, 1965 calories. Protein 50 gm., fat 165 gm., carbohydrate 60 gm. The last insulin was given on November 23rd. Following this the level of blood sugar rose promptly, although glycosuria did not occur.

November 28th. The patient went home to attend to business. Insulin therapy discontinued. Diet on discharge, 1350 calories.

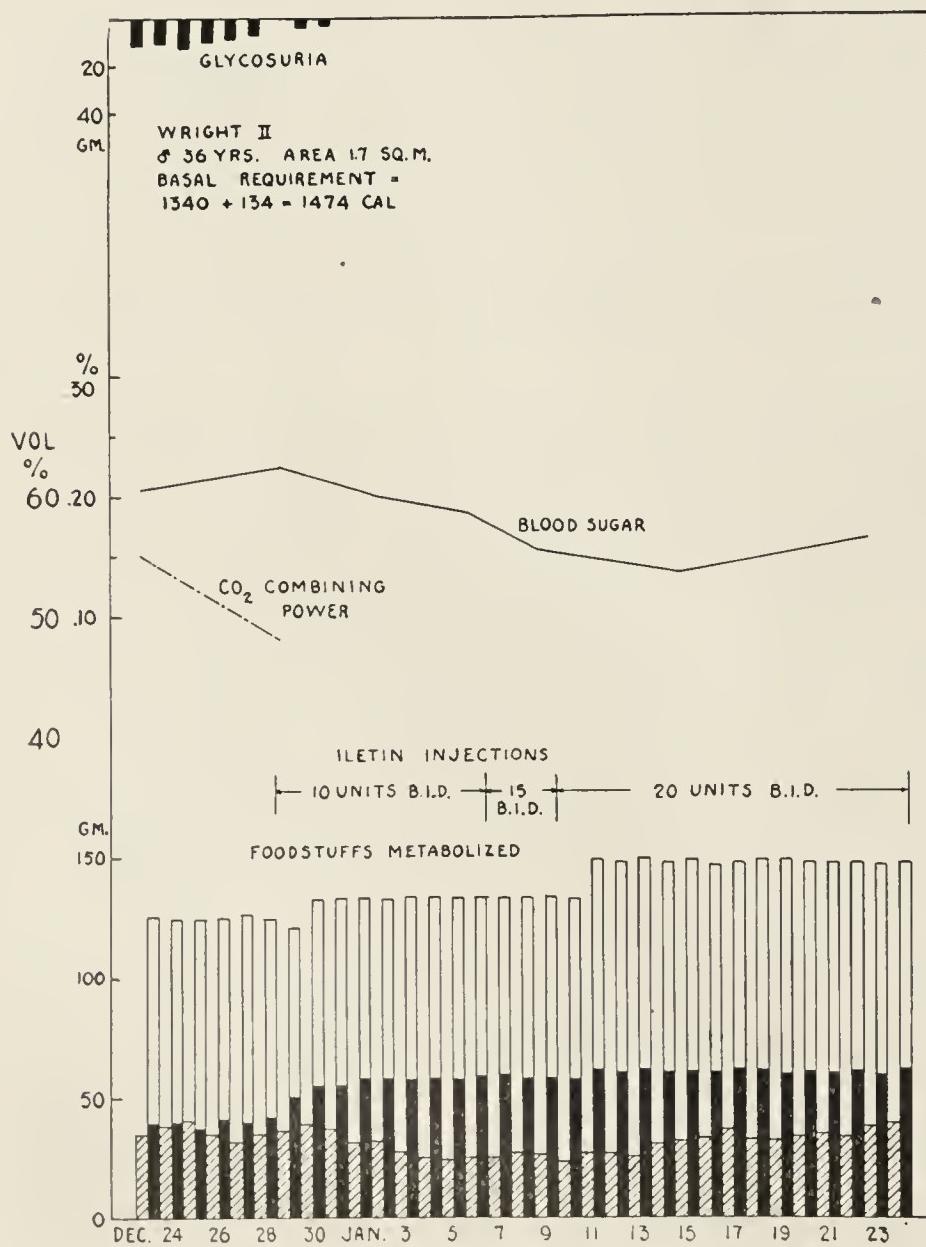


Fig. 6. Case 3. Symbols as in Fig. 1.

*Third admission, December 22, 1922:* On leaving the hospital the patient adhered to his diet as far as possible, but he was not very strict in his adherence because of sickness in his family. He began to have numbness of his feet. Sugar was found in the urine during the first week in December, only a trace being present. Large amounts of sugar were found on December 18, 1922, so that he returned to the hospital.

*Physical Examination:* Weight 57 kg. Slight impairment of resonance of both upper lobes as noted on previous examinations. There are no adventitious sounds indicating an active pulmonary process.

*Urine:* showed small amounts of acetone and moderate amounts of sugar. The CO<sub>2</sub> combining power was normal. Blood sugar=0.206%. Basal metabolism=1340 calories per diem. Basal requirement 1475 calories per diem.

*Course under treatment:* This is best shown graphically in Fig. 6.

December 23rd-28th. Diet, 1420 calories; protein 30 gm., fat 117 gm., carbohydrate 51 gm. Blood sugar rose slightly. The alkaline reserve diminished somewhat. Glycosuria persisted, 13-7 grams per diem.

December 29th.. Insulin (Iletin) 10 units b. i. d. Diet unchanged. Urine sugar-free. Last recorded acetonuria, FA/G =1.68, as calculated from patient's actual metabolism.

December 30th-January 6th. Diet, 1600 calories; protein 31 gm., fat 133 gm., carbohydrate 59 gm. Insulin 10 units b. i. d. Total 20. After two days no glycosuria. Fasting blood sugar level diminished slowly. No acetonuria, FA/G =1.38.

January 7th-9th. Diet unchanged. Insulin 15 units b. i. d. Total 30. Blood sugar continued diminishing. On January 9th insulin 20 units b. i. d., total 40, caused a mild hypoglycemic reaction.

January 10th. Diet increased to furnish 1830 calories. The same dose of insulin and same diet were continued until patient's discharge.

From January 10th to discharge on January 25, 1923, the weight remained about 60 kg. (57 kg. on admission). The blood sugar percentage was slightly above normal, 0.136-165%. The patient was allowed to go home. The same diet and the same dose of insulin were continued at home. Undoubtedly this patient needed a larger diet to carry on his regular work in the oil fields. Such a diet could be taken by him if larger doses of insulin were given. At the rate of five cents a unit the patient could not afford to take more than forty units per diem.

On a diet furnishing 1420 calories a dose of 20 units of insulin was required. With a diet giving 1830 calories 40 units were required. An increased utilization of 410 calories was obtained from 20 units of insulin, so that one may estimate a utilization of 20 calories per unit in this case.

**CASE 4 (abstract).** Haines, a laborer, 57 years of age, was admitted October 3, 1922, with the complaint of "sugar diabetes."

*Family history:* One brother had the same disease.

*Past history:* Poorly given on account of low intelligence. Headaches had been his most frequent trouble. These were described as "sick headaches." Colds in the head were frequent, and pains through the temples were complained of. He had lost twenty pounds in weight.

*Present illness:* Patient stopped work in February 1922 on account of weakness, which has been progressively worse since that time. He had frequent attacks of "misery" in the abdomen after eating, as well as cramp-like pains in the calves of the legs. There were "jumping headaches." His arms felt numb and sleepy.

**Physical Examination:** Mouth-breathing on account of nasal obstruction was noted. Patient was poorly nourished. The breath had a faint odor of acetone. Heart and lungs negative. The peripheral vessels were somewhat sclerosed. There was an area of localized tenderness in the mid-epigastrium. Otherwise the examination was negative. Temperature subnormal, pulse 100, respirations 20.

**Urine:** sugar abundant, acetone bodies present, a trace of albumin noted, but no casts or r. b. c.

**Blood:** Wassermann reaction negative. Non-protein nitrogen .041%. Roentgenogram of lungs: normal.

Basal metabolism: 1300 calories per diem.

**Course:** For the sake of brevity the metabolism tables are omitted. The data have been recorded graphically in Figs. 7

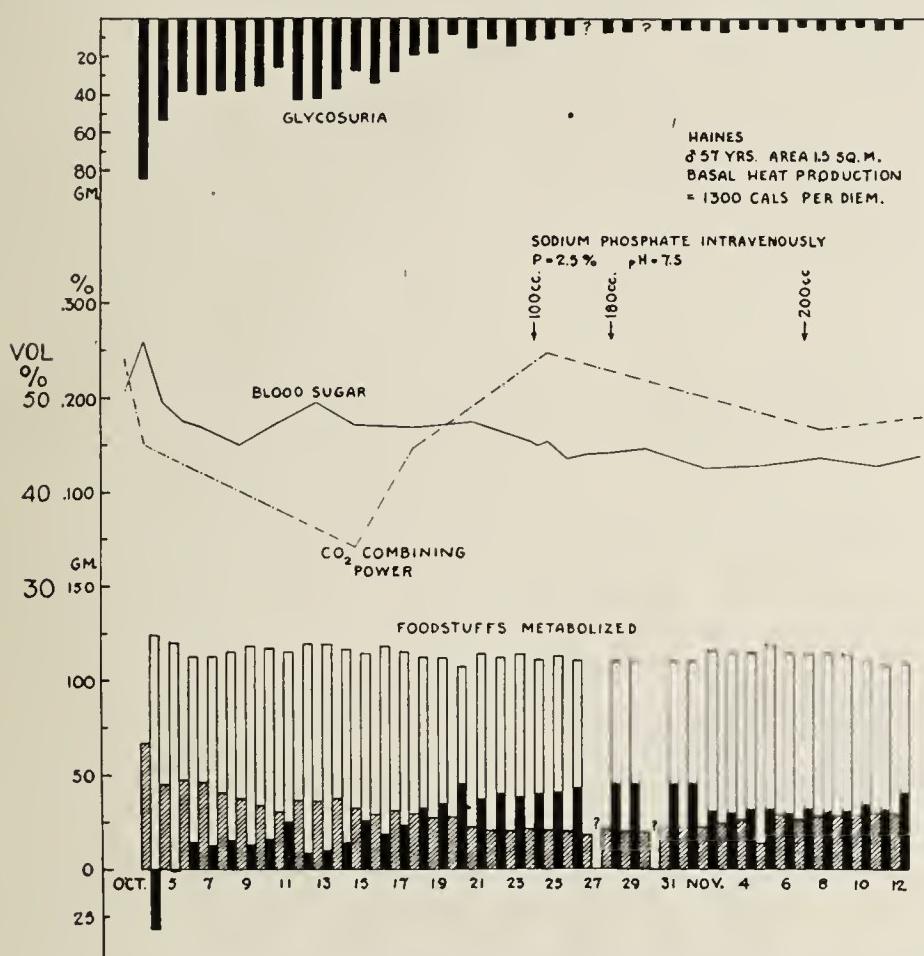


Fig. 7. Case 4. Symbols as in Fig. 1.

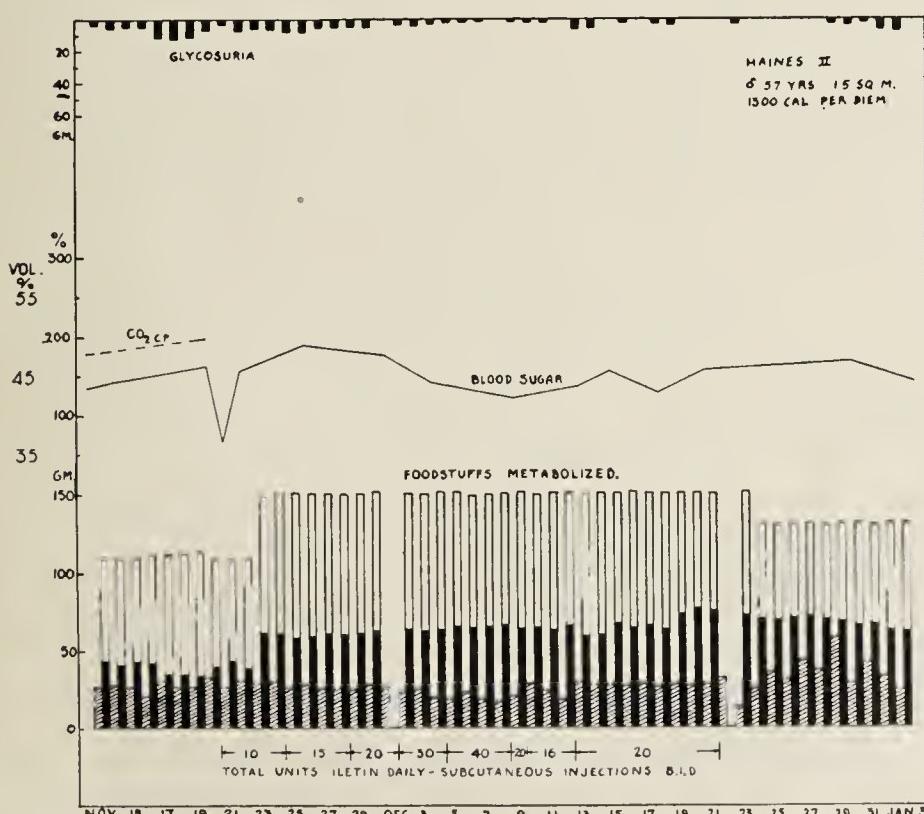


Fig. 8. Case 4. Continuation of Fig. 7.

and 8. Throughout the whole period of observation shown in Fig. 7, the diet furnished just enough calories to cover the basal heat production. There was a gradual decrease in glycosuria and in the degree of hyperglycemia, with a moderate increase in utilization of carbohydrate. Three intravenous injections of sodium phosphate (2.5% P., pH=7.3) produced no marked effect upon the level of blood sugar or amount of sugar excreted. This result is contrary to the claims made by Elias and Weiss.<sup>7</sup>

In Fig. 8 one may see the results of the use of insulin. There was initial hypoglycemia produced while the diet was still maintained at 1300 calories. Subsequently the diet was increased to 1800-1875 calories, and the dosage of insulin was increased to forty units per diem. With such a diet and such a dose normal blood sugar levels and a minimal glycosuria were noted. It may be estimated that each unit of insulin permitted the ingestion of 34 extra food calories. The patient was not suitable for a continuance of the use of insulin outside the hospital so that the dose was tapered down to from sixteen to twenty units per diem. These doses were not adequate. The use of insulin was discontinued, after which glycosuria was again increased.

The subsequent course of this case is unknown.

**CASE 5 (abstract).** Jarrett, a glass-worker, 58 years of age, admitted February 10, 1923, complaining of weakness, loss of weight, polyuria, polydipsia and polyphagia of five years' duration.

**Family history:** Unimportant.

**Past history:** Cerebrospinal meningitis at age of 3. Gonorrhœa at age of 23 years. Patient denies having had chancre or eruptions. He has been married for fourteen years but his wife has not been pregnant. Before the onset of illness he was somewhat obese—greatest weight 225 pounds.

**Present illness:** The onset was gradual in 1918 with weakness, loss of weight and a desire for sweets. A diagnosis of "sugar diabetes" was made. His diet, as prescribed by his physician, contained much meat, gluten and bran bread and green vegetables. On this diet he had glycosuria intermittently during five years. His symptoms have gradually increased and his weight decreased gradually to 150 pounds. During the four years prior to admission he had chills lasting for thirty minutes, which were followed by fever and profuse sweating. These chills occurred every two to three months, becoming more frequent of late. His legs seemed weak and he complained of some pains in them after exercise.

**Physical Examination:** Height 188 cm. Weight 68 kgm. Patient was of large frame, showing recent loss of weight. The important physical findings were: Early arteriosclerotic changes in retinae; emphysematous type of chest with signs of pulmonary emphysema; heart normal; larger arteries somewhat beaded and thickened; abdomen and genitalia normal; rectal examination negative except for old scar of healed fistula in ano; there were varicose veins in lower extremity; knee and ankle jerks sluggish; no definite sensory disturbance in lower extremities made out.

**Urine:** Normal except for heavy reduction with Benedict's solution. No acetonuria.

**Blood:** R. B. C. 5,140,000; W. B. C. 6,100. Haemoglobin 82%. Wassermann reaction positive. Hyperglycemia, normal CO<sub>2</sub> C. P.

**Roentgenogram of lungs:** Fibrosis of both lungs; dilatation of aorta; widened rib spaces suggesting emphysema.

<sup>7</sup> Elias, H., and St. Weiss: The action of the phosphoric acid ion on blood and urinary sugar. Wiener Arch. inn. Med., 1922, IV, 29.

*Phenolsulphonphthalein excretion:* 1st hour, 40%; 2nd hour, 2%; total 60%.

The patient was subjected to experiments on the effect of insulin and adrenalin, both separately and together upon the respiratory exchange and blood sugar curve. These will be reported in the succeeding paper of this series.<sup>11</sup>

Respiratory glucose tolerance tests were performed before treatment was inaugurated and again two weeks after treatment had been in progress. The results of these tests (Fig. 9) show a marked improvement in ability to oxidize glucose.

The course under treatment is shown graphically in Fig. 10.

From February 11th-15th the diet furnished 1800 calories (round numbers). The blood-sugar percentage continued high. Glycosuria was not marked, 11.4 gm. per diem. One injection of ten units of insulin intravenously on February 16th caused glycosuria to cease. The same diet was continued to February 21st. This diet covered his basal requirement.

From February 21st to March 8th the diet was increased gradually to 3000 calories. With injections of insulin once daily the urine was kept sugar-free. The morning blood sugar values were somewhat above the normal limit, probably be-

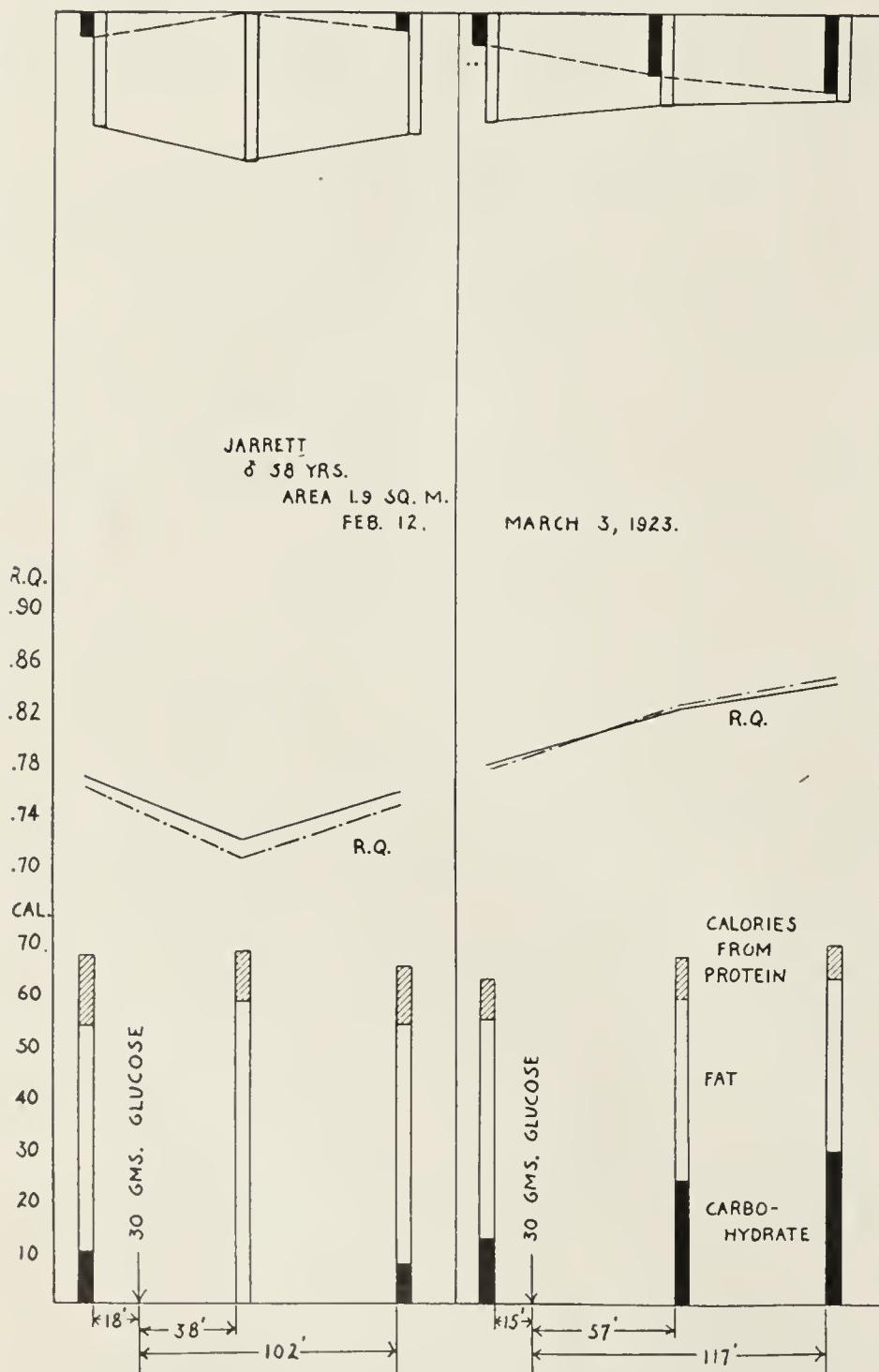


Fig. 9. Case 5. Data of respiration experiments showing improvement in power of oxidizing carbohydrate. Symbols as in Fig. 3.

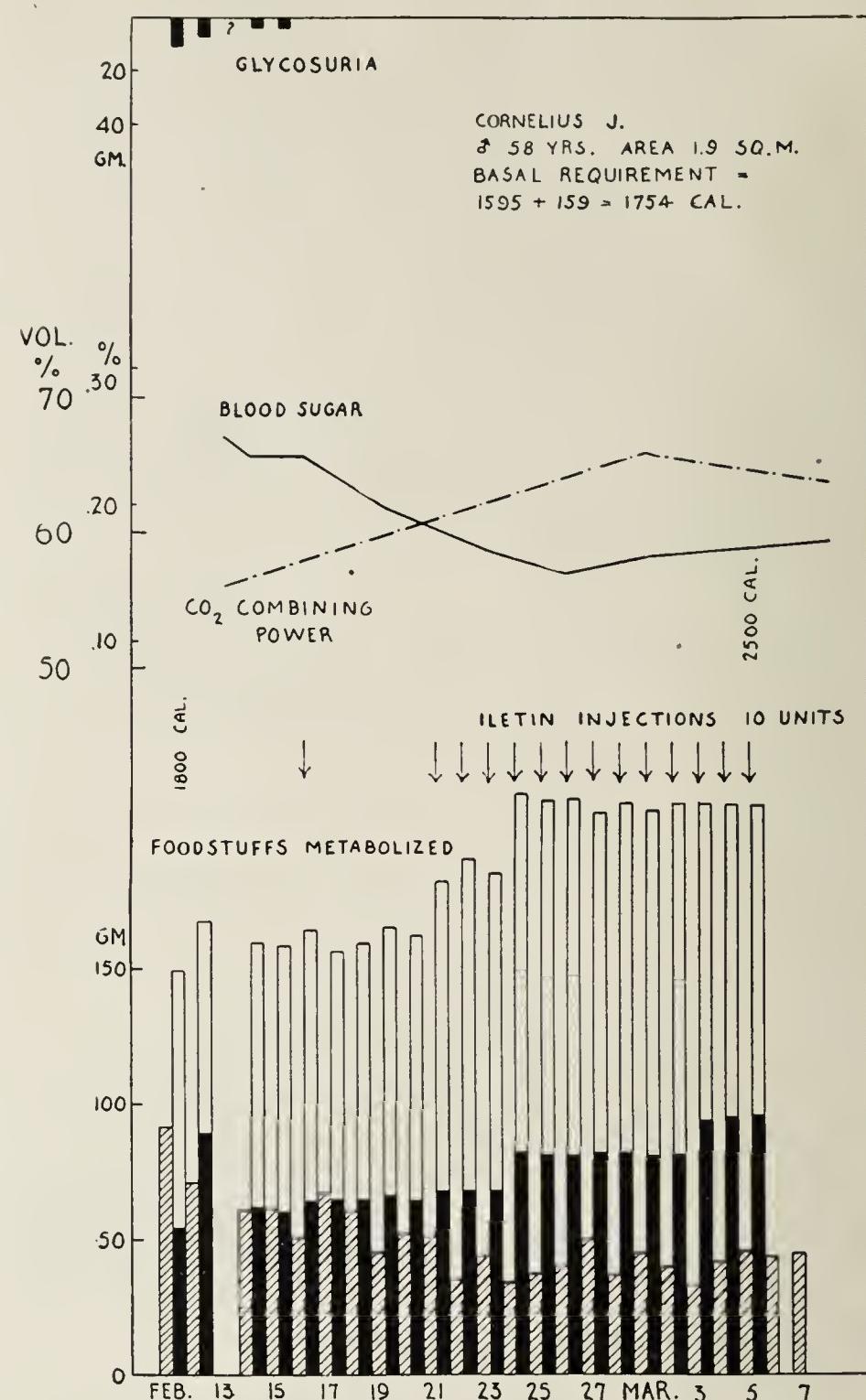


Fig. 10. Case 5. Symbols as in Fig. 1.

cause of the wearing off of the effect of an injection given at noon of the preceding day.

The patient felt much improved and able to resume work. The insulin treatment was continued under his family physician.

#### BASAL METABOLISM DETERMINATIONS

Date	Calories per hour	Calories per diem	R. Q.	Per cent of aver. normal
Feb. 12 . . . . .	67.9	1629	.769	94
Feb. 14 . . . . .	66.4	1595	.789	92
Feb. 16 . . . . .	65.6	1573	.749	91
Feb. 26 . . . . .	(57.3)?	....	.750	..
Mar. 3 . . . . .	62.9	1510	.777	87

It is estimated that 10 units of insulin daily permitted the utilization of more food, equivalent to 700 calories, or 70 calories per unit.

Calculating the total available glucose from the foodstuffs metabolized by the patient before and during treatment with insulin, one finds that the extra carbohydrate utilization was from 3.0-3.6 gm. per unit of insulin.

On April 24th this patient reported satisfactory progress. There had been no glycosuria with a diet of 95 gm. protein and 105 gm. carbohydrate with a variable amount of fat. The dose of insulin was unchanged. The patient gained weight and strength.

**CASE 6 (abstract).** Mrs. Desirée H., a housewife 50 years old, admitted January 8, 1923, complaining of diabetes mellitus, which she said had existed for 2½ years.

**Present illness:** The initial symptoms were thirst, polyuria and polyphagia with the finding of glycosuria. For ten months she regulated her own diet in accordance with the advice of her brother-in-law, a diabetic patient who had been treated at Battle Creek Sanitarium. She lost fifteen pounds in weight. In August 1921 she was a patient of Dr. F. M. Allen, Morristown, N. J., for three weeks. She left his care free from glycosuria, weighing 126 pounds. She continued sugar-free on the diet prescribed by Dr. Allen until February 1922, after which time glycosuria was constant. It is impossible to state definitely what her diet was during his interval. Presumably it furnished 1100 calories, but she "sometimes took a little more." She had some dyspnea at times, and occasionally a night sweat. On admission her weight was ninety-eight pounds.

**Family history:** Her father and one sister died of cancer.

**Past history:** General health always good. Diphtheria at age of eighteen years. Multiple arthritis at age of forty-two. Menopause since June 1922. Greatest weight 155 pounds; present, 98 pounds. Ideal weight, 127 pounds.

**Physical Examination:** Patient was emaciated, skin loose and dry. She was drowsy and there was a strong odor of acetone on her breath. Ophthalmoscopic examination showed early arteriosclerotic changes; media clear. Heart and lungs normal, except for a slight soft systolic murmur at aortic area. No marked sclerosis of brachial or radial arteries. Blood pressure 105/82 mm. Abdomen: the liver edge was felt just below the costal margin. The right kidney was palpable.

**Tendon reflexes:** In upper extremity they were sluggish; knee jerks and ankle jerks not obtained. No sensory disturbances detected.

**Urine:** showed large amounts of sugar, acetone and diacetic acid.

**Blood:** Sugar 0.301%. CO<sub>2</sub> combining power plasma, 26 volumes per cent. R. B. C. 4,280,000; Hemoglobin 93%; W. B. C. 5,000. Wassermann reaction: negative.

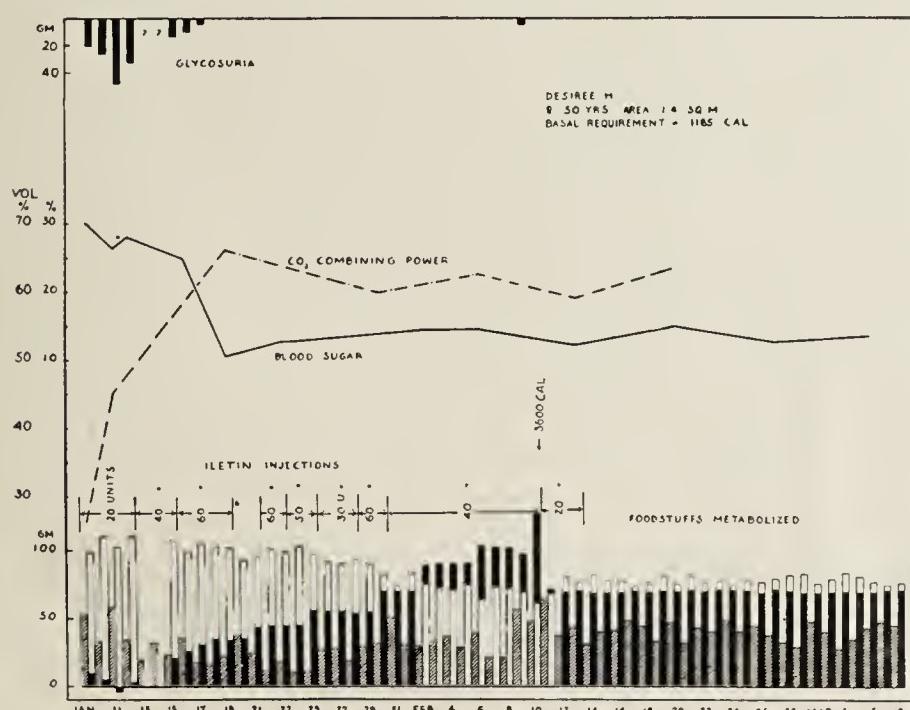


Fig. 11. Case 6. Symbols as in Fig. 1.

**Röntgenogram:** Chest—lungs clear.

**Basal metabolism:** Calories per diem 1087, 91% of average normal.

**Course:** With the above findings it was obvious that coma was impending and treatment with insulin was started at once. The course of the patient under treatment is shown graphically in Fig. 11.

On the first two days the diet was below maintenance, furnishing only 865-900 calories. Even with 20 units insulin per diem 25 grams of sugar were excreted.

January 11-20. The diet furnished 1000 calories; protein 30 gm., fat 80 gm., carbohydrate 35 gm. The doses of insulin were given subcutaneously at 8.30 a. m. and 5 p. m. The total dose was increased from 20 to 40 to 60 units. The last glycosuria was noted on January 17th. The blood taken before breakfast on January 19th showed normal alkaline reserve and sugar content.

The diet was rapidly increased from 1300 calories on January 21st to 3600 calories on February 10th. As mild hypoglycemic symptoms appeared, the dose was decreased gradually as the diet increased so that when 3600 calories were given in the diet the dose of insulin was only forty units per diem. The patient's weight increased from 87 to 102 pounds. In order to check this gain in weight the diet was reduced to 2000 calories and the dose of insulin decreased to 20 units once a day. Even this dose caused hypoglycemic symptoms and insulin was discontinued on February 15th. From February 15th to time of discharge from the hospital the patient continued to take a 2000 calorie diet (protein 50 gm., fat 165 gm., and carbohydrate 70 gm.) without any further injections of insulin. There was no recurrence of glycosuria and the blood sugar remained within normal values.

She was allowed to return home on this diet with provision for resuming insulin injections on recurrence of glycosuria.

One may estimate that an increased utilization of food equivalent to 2600 calories was made possible by the injection of 40 units of insulin per diem, or the equivalent of 65 calories per unit. This calculation takes no account of the patient's own increase in tolerance for food, incident to the readjustment of metabolism when glycosuria ceased and the acidosis was cleared up.

On April 25, 1923, the patient reported that she had been taking insulin since the second week after her discharge from the hospital. With a total of 14 units given in 2 injections daily, she remained sugar-free on a diet of 50 gm. protein, 165 gm. fat, and 70 gm. carbohydrate. She felt entirely well and engaged actively in her housework.

**CASE 7 (abstract).** Mrs. Grace W., aged 25, admitted February 6, 1923, complaining of polyuria, polydipsia, pruritus, furunculosis, weakness and loss of weight; duration two years. Onset occurred after the birth of her child two years before admission, with furunculosis of vulva and pruritus. The urine was not examined for sugar until January 1922. She was given a so-called "Allen Treatment," but it is an injustice to call the treatment by that name as the diets were given without reference to the presence of sugar in the urine, which was persistent for the whole year prior to admission. Her loss of weight was from 123 to 103 pounds. Her feet and ankles began to swell shortly before admission. She complained of "indigestion" and obstinate constipation. Amenorrhoea was complete for two years.

The positive findings on physical examination were: Marked undernutrition, dry skin with linear excoriations; furuncles on legs; heart and lungs normal; deep reflexes in the upper extremity were normal but in the lower extremity they were diminished and obtained only by reinforcement.

*Urine:* Greenish yellow, cloudy, specific gravity 1.042, sugar present, acetone and acetoacetic acid present, trace of albumin, and in the sediment were found hyaline, granular and cellular casts and many leucocytes. Urine culture: no growth.

*Blood:* R. B. C. 4,800,000; Haemoglobin 93%; W. B. C. 6,650. Normal differential count.

February 7th. Blood sugar 0.258%. CO<sub>2</sub> combining power 33.2 vols. per cent. Blood: Wassermann reaction, negative.

February 27th. X-ray Report: Chest—A number of dense circumscribed shadows in both lungs, which would indicate metastases from hypernephroma or some pelvic tumor.

There was nothing in the physical examination made following this report to indicate the presence of a tumor.

March 1st. Basal metabolism determined by means of CO<sub>2</sub> elimination, was three per cent below average normal, assuming a respiratory quotient 0.72. Basal metabolism of 1330 calories plus 130 calories equals 1460 calories, the basal requirement.

The course under treatment is summarized in Table II.

TABLE II.

Date	DIET				Dose of insulin	Urine sugar gm.	Blood sugar per cent	Blood CO <sub>2</sub> CP vol. %	33-59
	P.	F.	C.	Cals.					
Feb. 7-21 . . .	33	108	46	1325		47-12	0.258-0.231		
Feb. 22-28 . . .	33	108	46	1325	10	3-6	0.196		
Mar. 1-2 . . . .	33	108	46	1325	20	0	0.196- .099		
Mar. 3-5 . . . .	25	85	40	1055	10	0-2.4			
Mar. 6-8 . . . .	35	108	46	1325	10	0	0.110		
Mar. 9-13 . . . .	35	120	50	1460	10	0	0.110-0.170		
Mar. 14-19 . . .	45	144	60	1766	15	0	0.170-0.111	56.6	

The patient was discharged much improved in strength. The oedema and furunculosis had disappeared. Albuminuria and casts had disappeared. The low-grade nephritis probably was dependent on the pyodermia. The patient is continuing treatment at home on a diet of 1800 calories with 15 units of insulin given once a day by hypodermic injection. On April 29th she reported that she felt entirely well and that she was continuing sugar-free, with the same diet and dose of insulin as on leaving the hospital. Glycosuria, however, occurred if the injections were omitted for a single day.

CASE 8. Dorothy L. C. (Med. No. 48535) aged 16 years, was admitted December 14, 1922, in a semi-comatose condition with the odor of acetone on the breath and hyperpnoea. She had been known to have diabetes mellitus for six years. Treatment had been well carried out under good supervision for several years, but fifteen months before admission the child had been withdrawn from medical care and treated according to her father's ideas. A sweetish odor of the breath was noticed for three to four months before admission. For two weeks before admission she was short of breath. Gradually she became giddy, drowsy, and her speech was thick. She had difficulty in sleeping because of air hunger. On the day before admission she vomited several times and had a slight chill.

On admission, examination showed her to be in a semi-stuporous condition from which she could be roused to answer questions rationally. The face was flushed; respirations were deep and rapid; she was restless, tossing and moaning. An acetone odor filled the room in which she lay. Dehydration was evident in skin and low ocular tension and in slight polycythemia. Her skin had been blistered by a hot water bottle. Ophthalmoscopic examination showed normal retinæ and clear media. Heart and lungs were normal. Further examinations were negative. The urine contained sugar and acetone.

December 14:—

R.B.C. 5,850,000. Hemoglobin 90%. W.B.C. 15,700. Lipemia, globules stain with Sudan III.

		Blood sugar per cent	CO <sub>2</sub> CP plasma vol. %	Insulin given No. 67,141-725, 338
Dec. 14:	2 p.m.	.400	19.2	{ 10 units intravn. { 10 units intramusc.
	5 p.m.	.394	27.1	
	6 p.m.			50 units intramusc.
Dec. 15:	9 a.m.	.388	21.0	
	11.45 a.m.			50 units intramusc.
	2 p.m.	(500 c.c. normal saline intravn.)		50 units intramusc.
	4 p.m.			Temp. 102° F. Lungs clear.
	6 p.m.			Temp. 104. Few crackles heard at base right lung.
	8.30 p.m.			Patient suddenly became comatose, pulse rapid and weak, coarse rattle in throat, lips cyanotic; died in a few minutes.
	10.30			Blood from heart at autopsy contained 0.400% sugar.

The failure of insulin therapy in this case is not to be attributed to a lack of potency of the extract. The same lot of material was given to Irma P. (Case 47,840) from December 14th to December 20th. It required 50 units daily to keep Irma P. sugar-free, the same dose as had been required with previous lots of insulin. On withdrawal of the treatment hyperglycemia and glycosuria recurred promptly in the case of Irma P.

CASE 9. Harry B., aged 30 years, admitted February 24, 1923, complaining of diabetes mellitus, weakness and loss of weight. The onset of the disease was sudden, occurring while the patient was in active service in the trenches in France in July 1918. The initial symptoms were thirst, polyuria and polyphagia with headaches and gaseous eructations. He was discharged from the army in 1919, having been eating large quantities of candy and pastry. The frequent nocturnal urination interfered with his sleep. He was given a diet consisting chiefly of buttermilk and apparently his condition remained stationary for two years. At the Memphis General Hospital he was given a diet containing large amounts of protein. His weight and strength diminished rapidly. Since July 1922 he had been too weak to do anything. For three months prior to admission he had been in the Walter Reed General Hospital. At the time of admission to the Johns Hopkins Hospital his total weight loss was 50 pounds (normal weight 138 lbs.).

*Family history and past history:* Negative.

*Examination:* February 25, 1923: The positive findings were as follows: extreme emaciation; skin loose, dry, scaly, with xanthomata on arms and abdomen; mouth—dental caries and oral sepsis; eyes normal; no abnormalities found in heart and lungs; abdomen normal except for slight tenderness on palpation under the right costal margin; tendon reflexes normal in upper extremities but knee and ankle jerks were not obtained. Temperature, pulse and respirations were normal. The urine was of high specific gravity and contained sugar and acetone bodies.

February 26, 1923. Blood showed evidence of secondary anaemia. R. B. C. 3,576,000; W. B. C. 7,600; Hemoglobin

55%. Wassermann reaction: negative. Blood sugar 0.200%. Plasma CO<sub>2</sub> combining power 37 vols. per cent.

February 28, 1923. X-ray report. Chest—Tuberculous fibrosis right upper lobe with an area of bronchopneumonia at right base, and beginning changes in left upper lobe.

March 10, 1923. Four days after admission the patient began to have a low continuous fever with quick pulse and a cough which rapidly became quite productive. There was pain under the right costal margin. On examination there was impairment of pulmonary resonance over the upper chest, more extensive on the right side where dullness extended from the midscapula behind to the second intercostal space in front. On the left side there was dullness above the clavicle and on percussion of the clavicle itself, and at the apex of the left axilla. Over these areas the breath sounds were of tubular quality and many râles were heard. At the right base a leathery friction sound was heard over the sixth rib in the mammary line. No displacement of heart or trachea was made out. There was anisocoria, the right pupil being larger. Sputum contained tubercle bacilli. Vital capacity 850 c.c. Leucocyte count 5,200.

March 21, 1923. The x-ray showed extensive soft infiltration of the entire right lung and left lower lobe, suggesting a very active tuberculosis. In comparison with the plate made February 28th the tuberculous process had spread with remarkable rapidity.

March 24, 1923. X-ray Report: Humeri—examination of the humeri showed a curious mottling due to *localized* absorption of bone; in some ways this suggests a myeloma, but probably is due to some atypical non-malignant bone absorption. Tibiae: negative.

The course of the diabetes under treatment is shown graphically in Fig. 12, and the portion of his diet and metabolism record which covers the clearing up of glycosuria and ketonuria is shown in Table III. For the sake of brevity the rest of the table is omitted, since the data are recorded in Fig. 12.

In Table III calculations of the amount of fat oxidized daily during the period of marked ketonuria are at best rough approximations. The first difficulty encountered in these calculations was that of making an accurate estimate of the daily heat production. Basal metabolism determinations were made by means of the Tissot method, which showed a basal heat production of 1260 calories per diem. Additions were made to cover the elevation of metabolism in the presence of fever on the basis of data available in previous publications by McCann and Barr<sup>8</sup> which indicated that at a temperature of 104° F. the metabolism of a tuberculous patient is increased by 30% of the basal afebrile level. Allowance was made for the duration of fever during the twenty-four hours. An addition of 10% was made to cover the specific dynamic action of food and the slight activity of life in bed.

A further difficulty encountered was that of making proper allowance for heat lost from incompletely oxidized fat in the acetone bodies excreted in the urine and from the lungs. An approximate estimate of this loss was arrived at by calculating the urinary acetone bodies as B-hydroxybutyric acid, the heat value of which is 4.5 cal. per gram, and assuming that one-half of the urinary ketones were excreted as acetone from the lungs with a loss of 7.5 cal. per gram. (Landolt & Börnstein Tabellen). The oxidation of an extra amount of fat sufficient to cover this heat loss was also assumed. The validity of these

<sup>8</sup> McCann, W. S., and Barr, D. P.: Clinical Calorimetry, XXIX. The Metabolism in Tuberculosis. Arch. Int. Med., 1920, XXVI, 663.

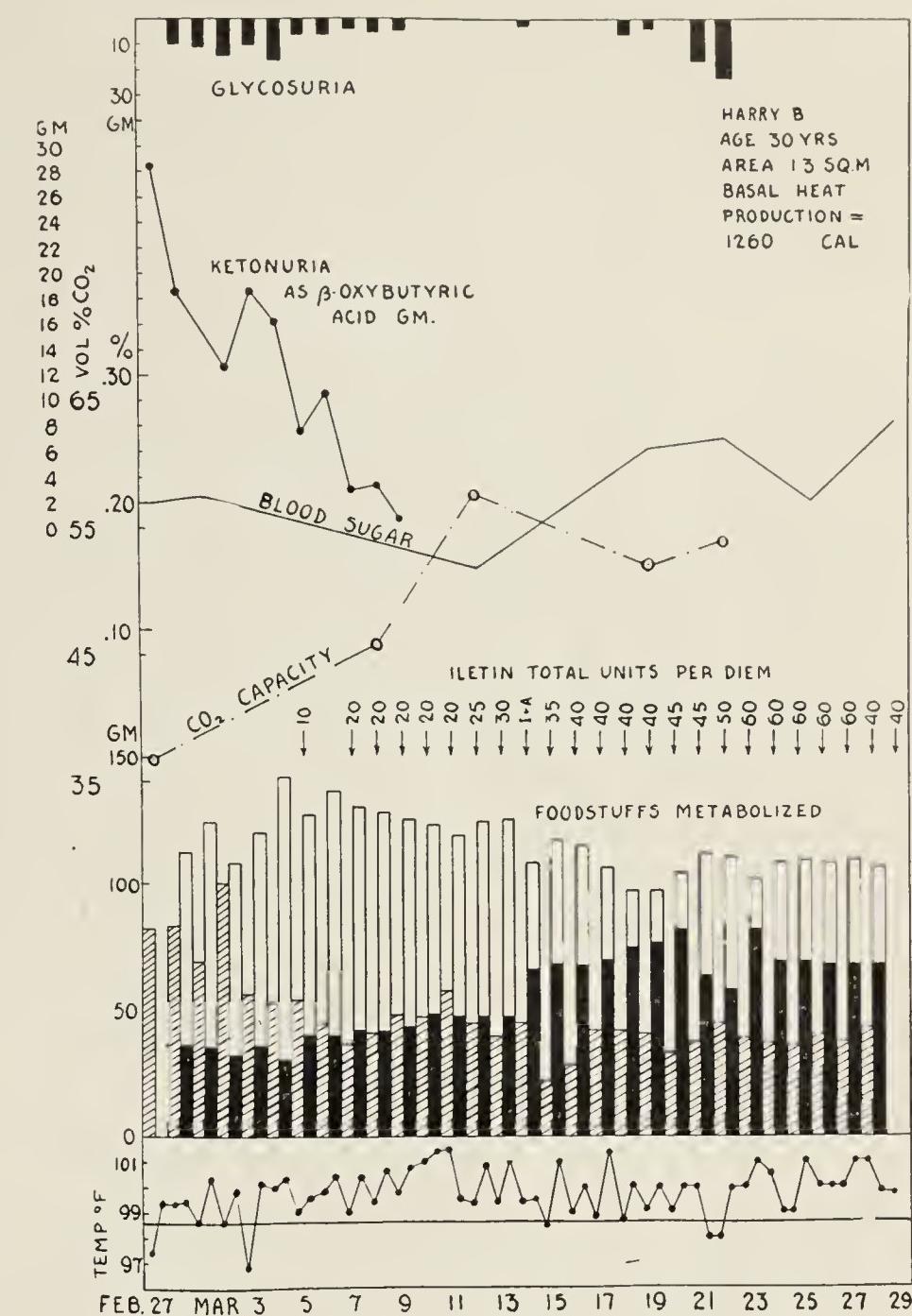


Fig. 12. Case 6. Symbols as in Fig. 1.

assumptions is attested by the finding of a fatty acid-glucose ratio of 1.6 at the time ketonuria ceased.

The effect of insulin on this patient was not very marked, owing, it is believed, to the interference of the fever of an active rapidly spreading tuberculosis. With a dose of twenty units of insulin daily the caloric intake was increased from 1320 to 1560 calories, twelve calories per unit, prior to February 13th. Under these conditions the fasting blood-sugar value was brought nearly to normal.

From the 16th to the 19th the diet furnished 2000 calories, but a dose of forty units daily failed to keep the fasting blood sugar level below .24% or to prevent slight glycosuria. For this period insulin furnished something less than seventeen extra calories per unit.

Subsequently with a diet of 2000 calories and an increased amount of insulin, sixty units were required to keep the urine sugar-free. The blood sugar percentage was abnormally high even with these doses.

The patient was transferred on March 29th to a National Soldiers' Home at Johnson City, Tennessee, at which place it was possible for him to continue the treatment with insulin (iletin).

On April 30, 1923, he was still living. Sugar-free urines were obtained, though the morning blood-sugar level was frequently elevated (0.170%). The diet and dose of insulin had not been changed.

TABLE III.

Case 9, Harry B., Age 30 years, Height 152 cm.,  
Weight 35.5 kg., Area L. 3 sq. m.

Date 1923	Vol. c.c.	URINE			BLOOD			DIET			FOODSTUFFS UTILIZED						Insulin units Total	
		Sugar gm.	Total acetone bodies B-oxy- butyric acid	N. gm.	CO <sub>2</sub> C.P. Vol. %	Sugar per cent	P. gm.	F. gm.	C. gm.	P. gm.	F. gm.	C. gm.	Av. G. gm.	Av. F.A. gm.	F/G	Cals. Req'd	Cals. Req.	Wt. kg.
Feb 27	3700	28.58	13.17	36.5	.200	31.1	108	47.1	82.3							1325	1390	35.40
28	3020	9.66	18.50	13.34		30.9	108	45.9	83.3	112	36.2	95.5	140	1.46	1318	1435	36.59	
Mar 1	3274	11.46		11.09	.204	30.2	108	46.2	69.3	124	34.7	87.5	144	1.65	1317	1485	36.64	
2	2343	14.65	12.45	16.10		31.1	108	46.2	100.6	108	31.5	101	143	1.40	1322	1450	36.73	
3	3380	10.35	18.85	9.01		30.7	108	45.9	56.3	120	35.5	80	144	1.80	1323	1390	37.40	
4	3360	16.46	16.03	8.38		31.1	108	46.4	52.4	142	39.9	84.5	152	1.80	1324	1500	38.23	
5	3940	6.85	7.72	8.60		30.8	108	47.0	53.7	127	39.1	82.9	139	1.67	1320	1500	38.09	
6	3700	6.84	10.51	7.12		31.1	108	46.4	44.5	136	39.6	79.0	143	1.81	1324	1530	38.32	
7	2680	4.23	3.04	5.89		30.8	108	46.0	36.9	130	41.8	76.2	134	1.76	1323	1530	38.42	
8	2700	5.40	3.52	6.55	45.7	55.3	122	46.7	40.9	128	41.3	77.8	135	1.73	1556	1530	39.55	
9	3380	4.87	0.75	7.60		56.0	122	47.8	47.5	125	42.9	82.9	134	1.61	1555	1530	40.38	
10	2620	0	1.70	7.52		55.9	122	47.9	47.0	123	47.0	86.5	132	1.53	1562	1530	40.38	
11	2520	0	0.84	9.05		55.6	122	47.5	56.6	119	47.5	92.2	133	1.45	1561	1530	41.18	
12	2470	0	0.76	6.94	57.6	.148	55.6	122	47.6	43.4	124	47.6	85.3	131	1.53	1553	1530	40.85
																		25 B.I.D. 10 and 15 units

CASE 10 (abstract). Ruth B., age six years, admitted to Harriet Lane Home on December 7, 1922, complaining of diabetes mellitus.

Family history unimportant.

Past history. She had always been a rather delicate child who gained slowly and was somewhat underweight for her age. Infections: pertussis at two years, parotitis at five years.

Present illness. Her present illness apparently began early in October 1922, when she began to complain of feeling tired and made the other children carry her home from school. At this time she had a poor appetite and no increased thirst or frequency of urination. At the end of October she had bronchitis, laryngitis and otitis media. Since then she had been in bed off and on. Her appetite had remained poor, but her thirst had increased and she had voided larger amounts than formerly. December 5th, 6th and 7th she ate nothing, drank very little water, and vomited frequently. Her temperature was sub-normal during this time.

Physical Examination, December 7, 1922. Temperature 97.3°. Weight 31 lbs., 3 oz. Height 41 inches. A pale child, who lay quietly in bed. She was very drowsy and paid little attention to her surroundings. Her eyes were sunken, her cheeks flushed, her skin loose and dry. She had moderate hyperpnoea and a marked odor of acetone to her breath. Although at the time she could be aroused sufficiently to answer questions, she later remembered nothing of coming to the hospital or of her first day there.

Urine: Sugar in large quantities. Much acetone and diacetic acid.

Blood: W. B. C. 6,960. Hgb. 65%. Blood sugar (15 minutes after first intramuscular injection of insulin) 0.268%. CO<sub>2</sub> combining power of plasma, 21.8 volumes per cent.

Because of the seriousness of her condition it was decided to use insulin at once. At 3.15 p. m. twelve units of insulin were given intramuscularly. By 6.00 p. m. the hyperpnoea was gone and she looked around and smiled. She continued to vomit and to refuse all food. At 8 p. m. normal salt solution was given per rectum. The morning of December 8th the

urine was sugar-free and had very little acetone or diacetic acid. The vomiting had ceased but she still refused to eat. She received six units of insulin. On December 9th she began to ask for food and a diet of P. 25, F. 54, C. 20, 686 calories, was begun. She received six units of insulin and her urine contained six grams of sugar. As her appetite returned the diet and the dose of insulin was gradually increased until on January 4th, when she was discharged, she was taking a diet of P. 48, F. 102, C. 38, 1300 cals. or 82 calories per kg., according to her weight at that time, 35 pounds 9 ounces. She was receiving eighteen units of insulin a day, ten in the morning and eight at night. There was no sugar in the urine after December 20th. At the time of discharge she was running around and playing, eating well and feeling well. It was arranged that she should receive insulin at home and return to the hospital from time to time for observation.

Subsequent course. At home she did very well but was always sleepy and cross in the evening. On January 7th her pupils became dilated and she complained of headache and inability to move her jaw. The next evening she was more drowsy, and repeatedly beat her head against the wall. The afternoon dose of insulin was reduced to five units. On January 22nd it was decided to give her 4/7 of her total calories at her noon meal and give her ten units of insulin before this meal. Her urine continued sugar-free and she continued to have hypoglycemic reactions until the dose was reduced to six units. Her weight on February 20, 1923, was 37 pounds, 12 ounces.

On February 26th she had otitis media with a temperature of 102° F. She lost her appetite, received no insulin and had sugar in her urine. Weight 35 pounds. March 13th she was again taking her 1300 calorie diet and six units of insulin and had gained back the weight lost. On March 15th she developed varicella. She would not eat all of her usual diet but took a diet of P. 36, F. 80, C. 30, 1014 calories. The dose of insulin was reduced to four units. There was sugar in the urine during the few days of fever but there has been none since. Since the varicella her appetite has not returned so that she is still

on the 1014 caloric diet. She weighed 37 pounds, 13 ounces on April 4th and felt quite well.

TABLE IV.  
Extra calories and carbohydrate rendered utilizable  
by 1 unit of insulin.

Case	Diet Calories Start	Diet Calories End	Increase	No. units per diem	Extra Calories per unit	Extra Carbohyd. per unit gm.
6	1000	3600	2600	40	65	2.85
5	1800	2500	700	10	70	3-3.6
2	1070					
	1250		40			
	1490		32			
	1490		24			
	1800		18			
	1800		25			
		310	(7)	44		
3	1350	1870	520	16	34	1.2
	1420	1830	410	20	20	0.85
4	1300	1875	575	16	36	1.6
7	1460			10		
	1766			15		
		306	.5)	61		3.6
9	1320	1560	240	20	12	1.05
	1320	2000	680	40	17	.88
	1320	2340	1020	60	17	.50
1	1180	1830	650	20	33	1.75

#### DISCUSSION

Study of the cases presented here can leave no doubt that the pancreatic extract "insulin" is a valuable therapeutic agent. In one of the ten cases it may be regarded as having failed completely to influence the disease in the dosage used. All of the other nine patients derived from the treatment some benefit, which was measured objectively in terms of lowered blood sugar values, decreased glycosuria, and in control of ketosis, as well as in the relief of symptoms.

It is quite apparent from the records that the effects of single injections of insulin are transient, so that in most cases two injections daily have been required. In some instances it has been possible to achieve the desired result with one dose daily. Gradual improvement in the general condition of the patient may make it possible to reduce the dosage in the course of time. A good illustration of this is seen in Adele H. (Case 2). Most interesting of all are those cases in which temporary cessation of insulin injections has been possible, as in Cases 1 and 6.

A striking variation in the extent of the response to therapy with insulin is shown in the cases. A number of factors which may be responsible for the variability of effect are at once apparent. The first concerns the

potency of the extracts. The methods of biological assay of insulin have been rather crude up to the present time. Comparison of the same doses of different lots of extract given to the same patient has revealed some unevenness in effects. This may be due to the method of assay, but it is fully as likely that it may be due to extraneous factors affecting the condition of the patient.

Wide variation in individual susceptibility to the effect of insulin was seen when the same lots of extract were compared in different patients. Dorothy L. C. (Case 8) showed no response to large doses of an extract of known potency, which was being used with success in several other severe cases. The calorie value of the extra food utilized per unit of insulin required to effect this utilization is shown in Table 4. These figures have been arrived at by comparing in each case the diet taken before the treatment with insulin with that which the patient could take while insulin was being given. The extra calories of food which patients were enabled to utilize per unit of insulin varied in these cases from 12 to 70 calories per unit.

In the same table, the extra glucose from all sources in the patient's metabolism has been compared with the dose of insulin which was required to render it utilizable. Again, a considerable variation in effect was noted, not only between different patients but on the same patient on different occasions. The amount of extra carbohydrate which was utilized per unit of insulin (all sources of glucose being considered) varied from 0.5 to 3.6 grams.

The reasons for this wide variation in individual susceptibility to the effects of insulin form an interesting subject for speculation and an important field for further investigation. Complicating infections, as in Cases 8 and 9, play an important rôle. Differences in the severity of the diabetes are shown rather in the total doses required to attain utilization of adequate diets than in the effects of a unit dose. It is difficult to evaluate improvement in utilization due to the readjustment of the whole metabolism which follows any period of freedom from glycosuria and hyperglycemia and the gradual building up of glycogen stores. There still remains to be considered the possibility of the existence of more than one type of diabetes mellitus depending upon the involvement of different mechanisms. There are many facts to suggest that this is true, though the proof is lacking. It is by no means conclusively proven that all cases of diabetes are due to lesions of the pancreas, or that in pancreatic diabetes deficiency of the pancreas is the sole factor. In the fatal case, Dorothy L. C., undoubted pancreatic lesions were demonstrated at autopsy. There was no distinct morphological proof of the failure of any extra-pancreatic mechanism of importance in carbohydrate metabolism, such as the liver, for although this organ was fatty and showed some evidence of "cloudy swelling," this was not more marked than

that frequently seen in the routine examination of livers at autopsy. It is conceivable that such a liver might fail to elaborate or to activate such an enzyme as was found to be essential for the *in vitro* action of insulin by the work of Forrest, Winter, and Smith.<sup>9</sup>

These writers found that the stereo-chemical changes produced in diabetic blood sugar *in vitro* by insulin required the presence of phosphates and an enzyme derived from the liver.

The existence of severe infection in the two patients who responded least to insulin (Cases 8 and 9) is of great significance. One may recall that infections more than anything else have been responsible for serious diminution of carbohydrate tolerance of diabetic patients previously under control; it is therefore not surprising that infections should be found to cause the action of insulin to be less effective and to require the use of larger doses.

The possibility of failure to control severe ketosis, especially in the face of complicating infections, makes it necessary to emphasize the necessity of combating ketosis by every means at hand. The valuable contributions to the knowledge of the mechanism of the ketogenic-antiketogenic balance of Shaffer and Woodyatt should not be brushed aside as no longer useful. The methods in use in this clinic for combating ketosis have been described in great detail in paper 2 of this series.<sup>3</sup> A further addition to this armamentarium has just been described by the writers,<sup>10</sup> regarding the therapeutic use of glycerol.

It needs further emphasis that the effects of insulin can be quantitatively measured in any given case. This necessitates a careful balancing of a measured diet against a measured dose of the drug. So far, in this clinic no hard and fast rule of procedure has been evolved, but each case has been worked out by a cautious "cut and try" method. One must study in each individual case not only what dose is required to enable the patient to ingest enough food to carry out the normal activities of life, but the division of doses and the time relationships between injections and meals need to be worked out carefully in order to avoid annoying and probably harmful periods of hypoglycemia. During the early stages of treatment these matters need frequent readjustment, and for this reason and for the purpose of the education of the patient, treatment is best initiated in a hospital.

Patients should be taught to weigh and prepare diets as before, and to examine the urine for sugar and acetone

<sup>9</sup> Forrest, W. D., Smith, W., and Winter, L. B.: On the change in the nature of the blood sugar of diabetics caused by insulin. *Jour. Physiol.*, 1923, LVII, 224.

<sup>10</sup> Hannon, R. R., and McCann, W. S.: Ketogenic-antiketogenic balance and the therapeutic use of glycerol. Paper read before the Am. Soc. for Clin. Invest., Atlantic City, N. J., April 30, 1923.

bodies. Treatment can then be carried out at home under the general direction of the family physician.

As a part of the training, a patient is allowed to have moderate hypoglycemia in order to acquaint him with the warning symptoms. He is instructed to keep always at hand a small amount of carbohydrate to use in case of need. Orange juice is recommended, but cane sugar is much more convenient. The use of adrenalin to counteract an overdose of insulin is not recommended, because it is too unreliable. This recommendation is based upon an extensive study of the effects of insulin and adrenalin on the respiratory exchange and blood sugar regulation in both normal subjects and diabetic patients made by R. S. Lyman and Elizabeth Nicholls in this clinic.<sup>11</sup> Other undesirable effects were noted.

In regard to dosage it has been the custom to seek for the amount, which, given subcutaneously, will render the urine free from sugar in an hour and one half and cause it to remain sugar-free for from twelve to thirteen hours, the patient being on a bare maintenance diet. The first dose of the day is generally given an hour before breakfast, the second generally about dinner time in the evening. If mild hypoglycemic reactions begin to occur, the diet is increased 200 calories every third day until a new tolerance limit is reached. If the diet is not adequate at this time, the dose is increased 10 units per diem. If reactions continue to occur after the patient has reached a diet which is adequate for all needs, the dose is reduced until no more reactions occur. Many patients continue to take more food than they need and in consequence tend to grow fat. This is felt to be highly undesirable. It may be overcome by increasing the patient's activity, or reducing the diet, or both, all changes being made gradually.

The psychic manifestations of hypoglycemia can not be adequately discussed at this time. They make an interesting subject for study. There is at the present writing in this clinic a young man who developed a delirium while undergoing a simultaneous treatment with insulin and starvation in another clinic. After a railway journey of several hours his blood sugar was found to be as low as 0.070% on his arrival at the hospital. The first treatment consisted in giving him a liberal diet, disregarding the diabetes. Under this treatment he improved. After a time it was again possible to control his diet accurately and to keep him sugar-free with insulin. He is now rational, with occasional mild relapses. An overdose of insulin brings out mental symptoms, the patient being blue and depressed. A slight tuberculous infiltration of both upper lobes of his lungs has apparently become quiescent, his nutrition is excellent, and a troublesome furunculosis has disappeared.

<sup>11</sup> Lyman, R. S., Nicholls, E., and McCann, W. S.: Proc. Soc. Exper. Biol. Med., April 18, 1923.

Economic problems have been frequently encountered. After leaving the hospital patients have been obliged to pay for the drug supplied by the manufacturers. It has been necessary in some cases to stop short of an ideal diet for the patient's needs in order to keep the cost of the extract within his ability to pay. At 5 cents per unit the cost to our patients has ranged from fifty cents to three dollars a day.

It is highly important that the dangers of overdosing with this extremely powerful drug be appreciated fully. Too much caution can not be used especially with regard to initial treatment. It is much better to make all changes in a diabetic patient's life slowly in order to allow time for readjustment. It is not advisable to give over 10 units as an initial injection, and then only when it is possible to observe the effects produced very closely. Carbohydrate in some readily utilizable form should be instantly available in case severe symptoms of hypoglycemia appear. Subsequent use of larger doses should be reached in easy stages.

#### SUMMARY AND CONCLUSIONS

Of ten representative cases of severe diabetes treated with insulin, nine derived some benefit, which was

measured objectively in terms of lowered blood sugar values, decreased glycosuria, improved oxidation of glucose, and in control of ketosis, as well as in the relief of symptoms. Wide variations were observed in responses of the different patients to unit doses of the drug. The extra calories of food which patients were enabled to utilize per unit of insulin varied from 12 to 70 calories. The amount of extra carbohydrate which was utilized per unit (all sources of glucose being considered) varied from 0.5 to 3.6 gm. In the presence of severe infection the action of the drug is apparently less effective. The importance of exact diet regulation is emphasized. Cautious methods of balancing the diet and dose gradually are illustrated. Attention is drawn to the dangers of hypoglycemia from overdosing. Prevention and treatment of this condition have been discussed.

Insulin is a very powerful and very valuable therapeutic agent from which most patients suffering from diabetes will derive benefit. It has failed therapeutically in certain cases in this series. It is in no sense a cure for diabetes so that it is highly important that other methods of influencing the disease favorably should not be neglected.

## THE TREATMENT OF ACTIVE INFANTILE TETANY WITH RADIATIONS FROM THE MERCURY VAPOR QUARTZ LAMP

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Huldschinsky<sup>1</sup> treated six children suffering with tetany by means of ultraviolet rays. Of these only one child was under 2½ years of age. The symptoms of active tetany disappeared in from four days to four weeks. In one patient the symptoms were aggravated by the treatment. No chemical studies of the blood were made.

It has previously been shown that the calcium concentration of the serum of rachitic children can be raised to the normal level by exposing the children to rays from the mercury vapor quartz lamp.<sup>2</sup> It has also been conclusively demonstrated that the calcium concentration of the serum is regularly low in cases of active infantile tetany, and that any agent which is capable of raising the calcium concentration to a level within 20 per cent of the normal will cure the active manifestations of tetany.<sup>3</sup> We have therefore exposed five children suffering with active infantile tetany to the rays of the mercury vapor quartz lamp in order to determine (1) whether such treatment regularly brings about the disappearance of symptoms, (2) what effect it has upon the calcium

concentration of the serum, and (3) whether healing of the accompanying rachitic process can be accomplished at the same time. Our results are summarized in the table. Clinical manifestations of tetany were present in every instance. Three of the patients had had convulsions before admission to the hospital. The electrical reactions and the calcium concentration of the serum showed the presence of active tetany. During treatment the diet in each case consisted of milk and farina. Exposure to the rays was carried out according to the following schedule:

Distance 18 inches
Intensity 9 amperes
1st day 5 minutes front and 5 minutes back
2nd day 10 minutes front and 10 minutes back
3rd day 15 minutes front
4th day 15 minutes back
5th day 20 minutes front
6th day 20 minutes back, and so on, alternating front and back. The time of any one exposure did not exceed 20 minutes.

Following raying the clinical manifestations of tetany disappeared in from three to seven days and the calcium

concentration of the serum increased approximately to the normal. It has previously been shown that similar results may be obtained when calcium chloride is administered. It should be emphasized, however, that the inorganic phosphorus concentration of the serum is not increased when calcium chloride is given. If the phosphorus of the serum is normal, the administration of calcium chloride produces a definite decrease of its concentration.<sup>4</sup> When, however, raying is employed, not only does the calcium concentration of the serum increase but the inorganic phosphorus concentration also increases. This treatment, then, not only brings about a subsidence of the manifestations of active tetany but also cures the accompanying rickets. The effect of the rays is just as striking with the negro child as with the white child.

In one instance the pH of the blood was determined before and after raying. No definite change could be made out.

The unpublished experiments of Wilkins, Boone, Holt and Orr, on one of our patients (Speipel) show clearly that the increase of the calcium and inorganic phosphorus concentration of the serum produced by raying is due to

increased absorption of these elements from the gastro-intestinal tract.

*Conclusions.*—In five consecutive cases of infantile tetany the symptoms disappeared after raying with the mercury vapor quartz lamp. In every instance the calcium concentration of the serum was increased by this treatment. The inorganic phosphorus concentration of the serum was raised to the normal level or above. Not only did the manifestations of tetany disappear but healing of the rachitic process was brought about by the treatment.

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## A NOTE ON THE BACTERIOSTATIC ACTION OF URINE AFTER THE INTRAVENOUS ADMINISTRATION OF MERCURO-CHROME TO NORMAL RABBITS

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Since the original presentation of Mercurochrome as "A New Germicide for Use in the Genito-Urinary Tract,"<sup>1</sup> its use has been extended, until recently the possibility of its intravenous administration has been considered. As the earlier experimental work was done in view of the use of mercurochrome as a local antiseptic, studies of its action intravenously have not been made, with the exception of the toxicity experiments mentioned in the first paper, and in the limited series of Piper.<sup>2</sup> In order to determine whether or not further clinical trial of mercurochrome intravenously is justified, its toxicity, antiseptic action, the mercury content of urine and the pathology of rabbits given from 1 mg./kg. to 10 mg./kg. intravenously are now being studied. In this paper, experiments on the bacteriostatic effect of the drug on urine are presented.

*Method.*—Preliminary tests before drug administration were as follows: weight, phenolsulphonephthalein excretion, examination of urine to exclude the presence of casts or albumin and of faeces to exclude diarrhoea. The drug was injected into the marginal ear vein, a freshly

prepared 1% solution being used. In this series single injections of 1 mg./kg., 2.5 mg./kg., 5 mg./kg. and 10 mg./kg. have been used, except in the case of Rabbit 11, which received two injections of 1 mg./kg. Urine was obtained by catheterization with aseptic precautions 5 minutes before injection and in general at intervals of 1, 2, 5 and 24 hours after injection. Of each specimen 0.1c.c. was plated to serve as a pre-inoculation sterility control. Of each specimen 2c.c. were put into a sterile tube and inoculated with 1 standard loopful of a 24-hour culture of *B. coli* in pH 7.6 dextrose broth. Thirty seconds, 1, 2 and 24 hours after inoculation, 0.1c.c. of the organism-urine mixture was removed, dilutions of 1:100, 1:1000, 1:10,000, 1:100,000 and 1:1,000,000 were made in 0.875% sodium chloride solution, 1c.c. of each dilution was added to melted pH 7.6 beef infusion agar, and plates were poured. After 48 hours of incubation at 37.5° C., the most suitable plates for counting were selected and the number of bacteria per c.c. was estimated. Controls on the identification of colonies obtained were made in every experiment. In this way, it

was possible to determine the number of organisms present at the time of inoculation and at the end of the chosen period of exposure. Having determined the action of normal urine, by the test before drug injection, it was possible to estimate the effect of the drug. In cases in which the normal urine was bacteriostatic, the increase in inhibition after drug injection could be shown. As inhibitive action was regularly obtained after injection, as compared with the controls, other factors remaining the same, we felt justified in attributing such action to the drug or its derivatives. The hydrogen-ion concentration of the urine was determined in every case in which a sufficiently large specimen was obtained and no marked or regular variation after injection could be

TABLE I.  
Injections of 1 mg/kg.

Rabbit	Time of catheterization	Time of removal of specimens for plating and number of bacteria per c.c. so obtained				
		30 sec. after inoculation	1 hour after inoculation	2 hours after inoculation	24 hours after inoculation	Uninoculated control
1.	5 min. before injection	Infinite	.....	.....	Infinite	0
	1 hr. after injection	Infinite	.....	.....	Slight Growth	0
	2 hrs. after injection	Infinite	.....	.....	0	0
	5 hrs. after injection	Infinite	.....	.....	Infinite	0
9.	5 min. before injection	9,800,000 (1.04)	10,200,000 (1.08)	10,600,000 (1.08)	26,000,000 (2.65)	0
	1 hr. after injection	8,100,000 (0.46)	3,800,000 (0.39)	3,200,000 (0.39)	10,800,000 (1.33)	0
	2 hrs. after injection	8,200,000 (0.45)	3,700,000 (0.5)	4,100,000 (0.5)	14,628,000 (1.78)	0
	5 hrs. after injection	9,500,000 (0.33)	3,200,000 (0.4)	3,800,000 (0.4)	.....	0
	24 hrs. after injection	8,600,000 (1.03)	8,900,000 (0.76)	6,600,000 (0.76)	.....	0
11-A	5 min. before injection	2,400,000 (1.20)	2,900,000 (2.08)	5,000,000 (2.08)	335,080,000 (139.61)	0
	1 hr. after injection	3,000,000 (1.06)	3,200,000 (1.1)	3,300,000 (1.1)	203,000,000 (67.66)	0
	2 hrs. after injection	2,900,000 (0.48)	1,400,000 (0.93)	2,700,000 (0.93)	107,000,000 (36.89)	0
	5 hrs. after injection	1,300,000 (1.76)	2,300,000 (1.69)	2,200,000 (1.69)	146,000,000 (112.3)	0
	24 hrs. after injection	1,000,000 (1.9)	1,900,000 (3.8)	3,800,000 (3.8)	4,400,000 (4.4)	0
11-B	5 min. before injection	2,000,000 (0.95)	1,900,000 (1)	2,000,000 (1)	66,000,000 (33)	0
	1 hr. after injection	1,700,000 (0.94)	1,600,000 (0.76)	1,300,000 (0.76)	44,000,000 (25.88)	0
	2 hrs. after injection	1,600,000 (0.56)	900,000 (0.62)	1,000,000 (0.62)	57,000,000 (35.62)	0
	24 hrs. after injection	1,400,000 (1)	1,400,000 (0.21)	300,000 (0.21)	50,000,000 (35.71)	0

found. The variations in drug action to be expected from the use of this method on account of the use of different animals would be paralleled in the clinical use of the drug.

*Experiments with 1 mg./kg. (Table I).*

Three rabbits were given this dose, one (Rabbit 11), receiving 2 injections, 3 days apart. In one case (Rabbit 1) the urine two hours after injection was bactericidal in twenty four hours, in marked contrast to the urine obtained 1 and 5 hours after injection. In one case (Rabbit 9) the urine before injection was bacteriostatic, as shown by the slight increase in 24 hours, but 1, 2, 5 and even 24 hours after injection the urine was more bacteriostatic than the control. The urine from Rabbit 11, following the first injection was most bacteriostatic 2 hours after injection, while after the second injection the maximum

TABLE II.  
Injection of 2.5 mg/kg.

Rabbit	Time of catheterization	Time of removal of specimens for plating and number of bacteria per c.c. so obtained		
		30 seconds after inoculation	24 hours after inoculation	Uninoculated control
7.	5 min. before injection	3,100,000	480,700,000 (157.)	0
	1 hr. after injection	5,800,000	22,260,000 (3.8)	0
	2 hrs. after injection	5,200,000	222,000,000 (42.7)	0
	5 hrs. after injection	6,500,000	512,152,000 (80.2)	0

inhibition was also found 2 hours after injection. In 4 cases, therefore, urine after injection of 1 mg/kg. was found to have bacteriostatic action, in 3 of these the inhibition being greatest two hours after injection, in 1 case 1 hour after injection. In 1 of these cases, the urine was bactericidal two hours after injection.

*Experiment with 2.5 mg/kg. (Table II).*

In this experiment, the urine reached its maximum inhibitive power one hour after injection, the inhibition gradually decreasing after that, although the specimen obtained 5 hours after injection showed approximately one half of the growth obtained from the control.

*Experiments with 5 mg/kg. (Table III).*

Three experiments were done with this dose. In 1 case (Rabbit 5) the urine 1 hour after injection was bactericidal in 24 hours. (See photographs.) In 1 case, Rabbit 10, the control was somewhat bacteriostatic, as shown by the slight increase in 24 hours, but the specimens obtained 1, 2, 5 and 24 hours after drug injection were much more inhibitive than the control. In the

TABLE III.  
Injections of 5 mg/kg.

Rabbit	Time of catheterization	Time of removal of specimens for plating and number of bacteria per c.c. so obtained				
		30 sec. after inoculation	1 hour after inoculation	2 hours after inoculation	24 hours after inoculation	Uninoculated control
5.	5 min. before injection	8,300,000	.....	.....	Infinite	0
	1 hr. after injection	4,700,000	.....	.....	0	0
	2 hrs. after injection	5,550,000	.....	.....	Heavy Growth	0
10.	5 hrs. after injection	9,750,000	.....	.....	Heavy Growth	0
	5 min. before injection	6,000,000	12,800,000 (2.13)	11,600,000 (1.83)	24,804,000 (4.13)	0
	1 hr. after injection	8,400,000	6,700,000 (0.79)	5,600,000 (0.66)	4,500,000 (0.53)	0
	2 hrs. after injection	9,200,000	7,400,000 (0.80)	6,200,000 (0.67)	4,400,000 (0.52)	0
12.	5 hrs. after injection	7,300,000	3,500,000 (0.47)	3,600,000 (0.49)	8,900,000 (1.21)	0
	24 hrs. after injection	10,600,000	7,600,000 (0.71)	7,300,000 (0.68)	.....	0
	5 min. before injection	590,000	550,000 (0.93)	790,000 (1.33)	147,000,000 (249.15)	0
	1 hr. after injection	690,000	700,000 (1.01)	900,000 (1.3)	65,000,000 (94.2)	0
	2 hrs. after injection	No Urine				
	5 hrs. after injection	220,000	150,000 (0.68)	141,000 (0.64)	5,200,000 (23.63)	0

third case (Rabbit 12), inhibition 1 and 5 hours after injection was shown.

#### Experiment with 10 mg./kg. (Table IV).

This experiment shows what the previous experiments have also indicated, that inhibitive action following drug-injection is not directly proportional to the amount of drug given. This animal, receiving what is usually the lethal dose, showed the most marked inhibitive action in urine obtained 5 hours after injection, as compared with maximum inhibition obtained 1 and 2 hours after injection with smaller doses. We have reason to believe that this is due to the fact that when a larger dose is injected, much of the drug is eliminated through the intestines, while with moderate doses, (in rabbits from 1 to 5 mg./kg.) the elimination is chiefly through the kidneys. This is confirmed by the fact that with the moderate doses given in these and other experiments, the animals remained free from diarrhoea, while with the dose of 10 mg./kg., in 2 cases, a violent diarrhoea has set in within 2 hours after injection, the faeces being highly colored with the dye.

**Summary.**—Inhibitive action of urine following intravenous injection of 1, 2.5, 5 and 10 mg./kg. of mercurochrome has been shown. The proportionate decrease, with maximum and minimum variations is shown in Table V. In two cases bactericidal urine has been ob-

TABLE IV.  
Injections of 10 mg/kg.

Rabbit	Time of catheterization	Time of removal of specimens for plating and number of bacteria so obtained				
		30 sec. after inoculation	1 hour after inoculation	2 hours after inoculation	24 hours after inoculation	Uninoculated control
8.	5 min. before injection	7,900,000	16,000,000 (2.02)	7,800,000 (0.98)	11,700,000 (1.48)	0
	1 hr. after injection	6,500,000	4,300,000 (0.66)	3,100,000 (0.46)	7,800,000 (1.2)	0
	2 hrs. after injection	7,100,000	3,200,000 (0.45)	3,100,000 (0.43)	5,724,000 (0.86)	0
	5 hrs. after injection	6,400,000	3,200,000 (0.5)	2,900,000 (0.45)	4,707,000 (0.73)	0
	24 hrs. after injection	9,500,000	8,000,000 (0.842)	13,300,000 (1.4)	.....	0

tained, one after a single injection of 1 mg./kg., and one after a single injection of 5 mg./kg.

**Conclusions.**—The clinical trial of moderate intravenous doses of mercurochrome is justified from the point of view of bacteriostatic action, in *B. coli* infections of the urinary tract.

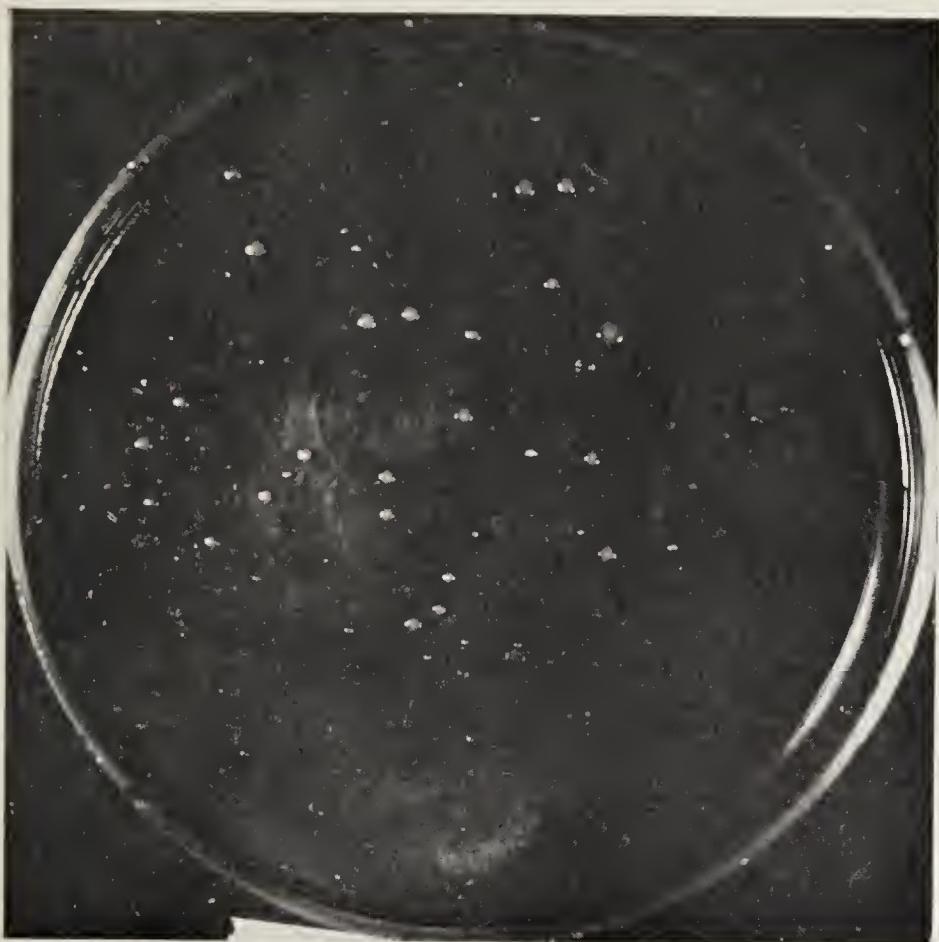
TABLE V.  
Summary showing relation of number of bacteria to those present at time of inoculation (taken as 1), compiled from averages of Urines before and after drug injection.

	1 hour after inoculation			2 hours after inoculation			24 hours after inoculation		
	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Before injection (Normal control)	2.13	0.93	1.37	2.08	0.98	1.38	249.15	1.48	83.86
1 hour after injection	1.06	0.46	0.82	1.30	0.39	0.77	94.2	0.	24.3
2 hours after injection	0.80	0.45	0.54	0.93	0.43	0.63	42.7	0.	16.9
5 hours after injection	1.76	0.33	0.74	1.69	0.4	0.73	112.3	0.73	43.61
24 hours after injection	1.9	0.71	1.09	3.8	0.21	1.37	.....	....	....

These figures were obtained by dividing the number of bacteria per c.c. at the end of the exposure periods of 12 and 24 hours after inoculation, by the number of bacteria per c.c. present 30 seconds after inoculation.

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Single dose of 5 mg/kg. See Table IV

Plate 1-A.—Urine before administration of drug. Plate containing 1 c.c. of a 1:100,000 dilution of urine, made 30 seconds after inoculation.

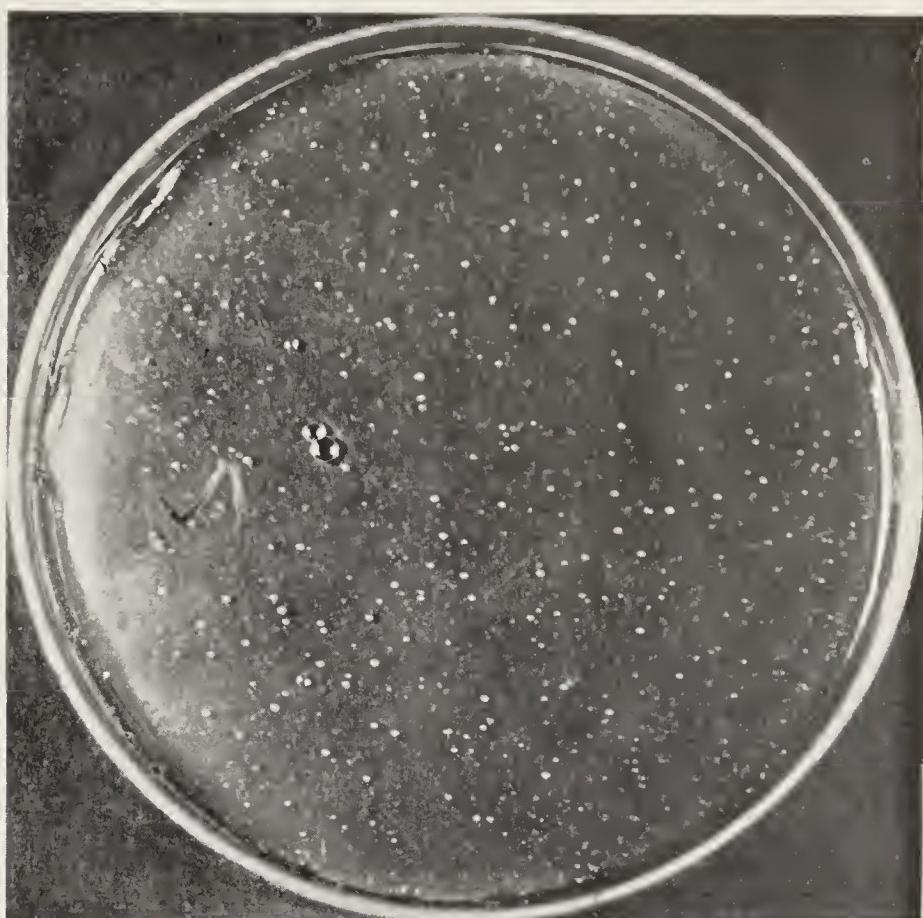


Plate 1-B.—Urine before administration of drug. Plate containing 1 c.c. of a 1:100,000 dilution of urine, made 24 hours after inoculation, showing an increase in the number of organisms.



Plate 2-A.—Urine 1 hour after administration of drug. Plate containing 1 c.c. of a 1:100,000 dilution of urine. Made 30 seconds after inoculation.



Plate 2-B.—Urine 1 hour after administration of drug. Plate containing 0.1 c.c. of urine, made 24 hours after inoculation, showing no organisms.



## THE DESTRUCTION OF BACILLUS RADICICOLA BY THE CONNECTIVE-TISSUE CELLS OF THE CHICK EMBRYO IN VITRO

By M. R. LEWIS

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It has long been known that various kinds of cells are capable of ingesting and destroying many different organisms (Metchnikoff, 1901). Certain investigators have associated this phenomenon with definite enzymes, thought by some to be located within a vacuole surrounding the ingested particle; by others, in the white blood cells and macrophages. Experiments carried on by workers in this laboratory, using all sorts of foreign bodies in connection with tissue-cultures, have led to the conclusion that the protoplasm of connective-tissue cells possesses a very active digestive power which is capable of breaking down foreign bodies, such as melanin pigment granules, white of egg, red blood-cells, avian tubercle bacilli, *bacillus tumefaciens*, and various other organisms. Since it was not known whether any of the organisms used are capable of living under the conditions which exist in living protoplasm, it was thought of interest to observe how the connective-tissue cells would treat such an organism as *bacillus radicicola*, which is supposed to live and multiply inside certain cells of the legumes (Dawson, 1900).

A strain of *B. radicicola* isolated from the soy bean and another from the red clover were kindly sent to me by Dr. Lewis Leonard of the Department of Agriculture. Over a hundred 24-hour cultures of the connective-tissue cells of chick embryos (6 to 9 days' incubation) were inoculated with the organism and observed. The tissue was explanted into Locke-Lewis solution (85 cc. of NaCl 0.9% plus KCl 0.042% plus CaCl<sub>2</sub> 0.025% plus NaHCO<sub>3</sub> 0.02% plus 15cc. of chicken bouillon plus 1% of dextrose) having a hydrogen-ion concentration of 6.6. After 24 hours a heavy suspension of the organism was prepared in the same medium and a drop placed on the tissue-culture, which was sealed and placed upside down in the incubator for one to four hours, and then bathed with sterile Locke-Lewis solution to remove the excess organisms.

A solution of neutral red (1-50,000) was sometimes used to stain these preparations, and a few were stained with janus green (1-100,000). Each of these dyes was first dissolved in the nutrient medium and then a drop of this was placed upon the culture and observed immediately.

Although both strains of *bacillus radicicola* were motile and the one from the soy bean, according to Dr. Leonard, has two polar flagella, neither the organism

nor the cell made any effort to approach each other. An organism often hit against the cell or remained beside it, while the cell sent out and withdrew processes in any and every direction, sometimes even moving against the organism without ingesting it. On the other hand, many organisms entered the cell, but this occurred only when the bacillus became so placed by chance that its entire length touched the cell. The behavior of this organism toward the cell and of the cell towards the organism was the same as that described in the case of avian tubercle bacilli by Smith, Willis and Lewis (1922). Organisms that touched a cell throughout their entire length usually remained motionless for a period of time, and then it became evident that they had entered the cell, because they began to undergo that peculiar movement exhibited by all foreign bodies within the cells of tissue-cultures (Smith, 1921). The movement was not at all like the motility characteristic of these organisms, neither did it resemble Brownian movement, but was a slow travelling to and fro between the periphery of the cell and the nucleus. Sometimes the organism travelled more or less around the nucleus on the side away from the centrosphere, often closely encircling it. Not all of the organisms that remained quiet against the cell entered the cytoplasm. Such a bacillus occasionally gave a jerk or two, became again motile and moved away from the neighborhood.

When a heavy suspension of the strain from the soy bean was placed upon a culture, the whole free surface of the cells became covered with the bacilli arranged at right angles to it, twisting and jerking about as though their flagella were stuck to the cell. None of these entered the cells unless first drawn flat against one.

The number of organisms that entered a cell depended entirely upon chance; that is, upon the number that happened to come in contact with it in the proper manner. If the suspension was uneven, one cell might be covered with organisms while an adjoining cell might be entirely free from them.

Those organisms that did not become stuck to the cells or to the coverglass gradually became less motile and after 24 to 48 hours sank to the bottom of the hanging drop. During this time they multiplied slowly, but never increased in number sufficiently to kill the tissue-culture. If the culture was left inverted after it was bathed to free it from the excess of bacilli, the organisms

almost disappeared, because as they became less motile most of them fell upon the cells and were destroyed.

It may be that *B. radicicola* is able to survive within the plant cell but it certainly is not capable of living within the embryonic connective-tissue cells. These cells must possess great oxidizing power, for they took in and destroyed many organisms within a short period of time ( $\frac{1}{2}$  to 2 hours). When a single cell was kept under observation, the actual number of organisms destroyed could be determined. One such cell had ten bacilli moving in the cytoplasm at the beginning of the observation and during the two hours it was watched it destroyed these and thirteen more. All that could be seen of the process of destruction was that the bacillus, which at first moved about in the cell as a highly refractive body, became thinner and thinner and less and less discernible, until after thirty minutes or more it could be located only by a granule at each end. These granules continued to be moved about, keeping the same distance apart for some time and then for a while they were no longer so definitely spaced, and finally disappeared altogether. In some cells these granules remained for so long a time that it seemed as though they might be spores, but in no instance were they seen to form new organisms, even after the death of the cell.

The bacilli usually faded away without any accompanying vacuole. They were not taken into vacuoles, neither did a vacuole form around them during the course of their destruction. In fact, there seldom were any vacuoles in the cells during the breaking down of the organism and never an accumulation of them as in cultures containing *B. typhosus* (Lewis, 1920).

In order to determine what would happen if vacuoles were present, a series of 50 cultures were explanted into a solution from which dextrose had been omitted, as it has been shown that cells growing in such a medium rapidly become full of vacuoles (Lewis, 1922). These cultures were inoculated in the same manner as the above. The organism entered the homogeneous cytoplasm and frequently, though by no means always, a vacuole later formed around it. In some cases a single vacuole formed around two or more organisms, which then became clumped. Where it was possible to compare the time necessary for the destruction of an organism in the homogeneous cytoplasm with that of one in a vacuole, it was found that this took place no more rapidly in the vacuole than in the cytoplasm; instead, digestion was usually slower in the vacuole.

Cells growing in solutions containing dextrose frequently divided, even though full of organisms, and this multiplication always took place by mitosis. One of the interesting occurrences in this phenomenon was that during mitosis the bacilli frequently became arranged each with its long axis parallel to that of the spindle in a belt around the chromosome plate, and as the cell divided into two about half the number of bacilli passed

across into each daughter-cell. This behavior suggests that the similar arrangement of mitochondria in certain dividing cells may be due to the physico-chemical factors involved in mitosis and not to the activity of the mitochondria themselves.

#### VITAL DYES

When a preparation in which the cells had ingested *B. radicicola* was stained with brilliant cresyl blue 2b (1-50,000), most of the organisms within the cells remained entirely uncolored; a few, however, were slightly colored or had a small purple granule at one side or within the bacillus. The stain depended upon the state of the cell, for if the dye solution was first reduced by boiling it with zinc, then the organism within the cells frequently became pink or lavender with more deeply stained granules.

When cells containing organisms were stained with a solution of neutral red (1-50,000), the cytoplasm, nucleus and most of the organisms within the cell remained colorless, while a few organisms became outlined with a faint red band and others contained a pink granule. Certain granules native to the cytoplasm became a bright red. The organism was destroyed regardless of whether it was colored or not, but the breaking down of all organisms went on much more slowly in stained preparations. At first it was thought that the pink color was an indication that a vacuole was present around the organism, but later it seemed to be merely an indication of the amount of oxidation going on.

Janus green also stained a few of the organisms inside the cells as well as all the mitochondria, but only in a few of the cells and then only one or two of the organisms within a cell; most of them remained uncolored. The stained organism was sometimes a faint diffuse blue; in other instances only certain granules in the organism were blue. On the other hand, those granules set free toward the end of the digestion of the organism nearly always were a deep blue. The color was never safranin (reduced janus green). Janus green is toxic to the cells and brought about their death before any further digestion of the organisms occurred. When cells containing stained organisms died, the color did not leave the organism as it did the mitochondria, but remained for some time after the whole culture was dead. Organisms outside the cells did not become colored with brilliant cresyl blue, neutral red or janus green. Just what the staining with these dyes indicated is impossible to determine at the present time, as the whole phenomenon is involved in that of reduction and oxidation of the dye itself.

#### THE CLASMATOCYTES

The foregoing observations apply only to the fibroblasts and not to the wandering cells (tissue macrophages of Evans and others) of the connective tissue.

Just what percentage of clasmatoctyes occurs in tissue-cultures is impossible to state, but there are fewer of these cells than of fibroblasts. When grown in this particular medium, these cells exhibited great activity in sending out and withdrawing processes, but not in locomotion. They migrated out slowly and were seldom found along the periphery of the growth. They did not tend to progress in one direction, as did the fibroblasts, but moved around over the fibroblast or even back toward the explant but never migrated far away. Smith (1921) and Smith, Willis and Lewis (1922) have shown that while clasmatoctyes do not go about gathering up avian tubercle bacilli or melanin pigment granules, nevertheless, these cells ingest many more foreign bodies than do the fibroblasts. This was true in regard to *B. radicicola*. The contact with these cells and the bacilli took place purely by chance. When the suspension was uneven, one clasmatoctye was free from organisms, while another was loaded with them, but given the same chance infection as the fibroblasts, the clasmatoctye took in many more organisms. The organism entered this cell much more rapidly than into the fibroblast, but regardless of its many twisting, sheet-like processes the manner of entrance of the bacilli was the same. *B. radicicola* did not seem to be destroyed as rapidly in the clasmatoctye as in the fibroblast. This may be because it contained so many, or perhaps because the cell was so thick that the entrance of more organisms into it was not detected. One particular cell contained about 80 bacilli when placed under observation; at the end of 24 hours it still had about as many, although the organisms had by this time become granular; after 48 hours most of the bacilli had been entirely digested but some still remained. Clasmatoctyes more frequently contain vacuoles than do the fibroblasts, but usually more organisms were observed in the homogeneous cytoplasm than in vacuoles. The region of the centrosphere seemed always to be free from bacilli, although they radiated thickly around it. Sometimes the clasmatoctyes were so full of organisms that the only clear places were the nucleus and the centrosphere.

When cultures died, as they usually did after 5 or 6 days, there were seldom any organisms left in the fibroblasts, but quite frequently there were some in the clasmatoctyes and in several instances these moved as though alive.

#### DISCUSSION

The ingestion and destruction of these organisms, which seems such a simple performance as one watches it in tissue-cultures, certainly emphasizes again the remarkable structure of the living cell. The motility and the gradual fading away of the bacilli indicate, as has been shown by Mathews (1921), that intense chemical changes are taking place, even though we cannot see them. For this reason it is impossible to gain any clear insight

into the behavior of a living cell by a study of an artificial protoplasm such as that used by MacDougal. What agar mixture could reduce neutral red while permitting certain bodies within it to become colored by the dye, exhibit movements of locomotion as well as currents within itself, and digest a number of bacilli, some of which may be slightly colored by the dye? The fact that foreign bodies find their way into the cell and the manner of their entrance bring up the question of the cell membrane. One interesting point in regard to this structure that was brought out by a study of the destruction of *B. radicicola* is that, while many of the organisms were seen to be digested within the cell, no changes were ever observed in those that lay against the cell.

The question as to whether the mitochondria may not be organisms living in symbiosis with the cell has been raised from time to time. Only recently Cowdry and Olitsky (1922) attempted again to show the fallacy of this theory by pointing out the difference in reaction of the mitochondria and certain organisms when stained with janus green. While I agree with what these observers state in the discussion of this question, nevertheless, I am forced to take exception to the method employed by them, because the living cytoplasm is such a remarkable substance that it is impossible to compare what takes place within it with what happens outside. In other words, if Cowdry and Olitsky wished to compare the staining of the mitochondria with that of an organism, it seems to me that the organism would have to be inside of the cell. Most of the organisms experimented with in this laboratory do not stain with janus green inside the cell. *B. radicicola* is somewhat different in this respect from the avian tubercle bacilli, *B. tumefaciens*, *B. typhosus* and others, as it does occasionally become somewhat colored with janus green. The mitochondria themselves stain with janus green only when the cell is alive and not always then, for it is a simple matter to remove all color from these bodies by bathing the cells with a weak solution of potassium cyanide. Under such conditions the janus green is not removed from the mitochondria but only rendered colorless and the color returns at once when the cyanide is washed off with a solution free from dye.

Pathologists usually imply that most phagocytosis takes place by means of the leucocytes, often not taking into consideration the activity of the connective tissue. An infection in a tissue-culture is by no means comparable to that in an animal; nevertheless, it is interesting to observe what a single type of cell is able to accomplish for its own protection, even though entirely removed from the numerous other conditions which accompany an infection in an animal, such as heat, leucocytosis, respiratory changes and the various factors involved in immunity. The great number of organisms and other foreign bodies which the connective-tissue cells are able to destroy in tissue cultures suggests that these

cells may frequently play a part in phagocytosis, but because of the rapidity with which the ingested organism is destroyed it is seldom observed in the animal body. The power of digesting the organism seems to be in the homogeneous cytoplasm and not in specific granules or vacuoles.

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## THE INCREASING SIGNIFICANCE OF PERMEABILITY-PROBLEMS FOR THE BIOLOGICAL AND MEDICAL SCIENCES

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(*The Charles E. Dohme Memorial Lectureship. First Course.*  
Oct. 10, 11, 12, 1922)

#### LECTURE II

1. *Experimental evidence of the change of permeability (vital permeability) to electrolytes.*
  - (a). Permeability of red blood corpuscles under the influence of physiological amounts of CO<sub>2</sub> (1892). Significance in physiology and pathology. Experiments of Girard.
  - (b). MgSO<sub>4</sub> and intestinal epithelium (1898).
2. *Experimental evidence of the change of permeability to anelectrolytes (glucose).*
  - (a). Permeability of the glomerular epithelium to glucose.
    - $\alpha$  Effect of free calcium ions, also in other cases (red blood corpuscles, spasmophilia, stomach, rectum, œdema and capillary contraction).
    - Estimation of Ca-ions.
    - $\beta$  Effect of glucose itself. Renal tolerance.
    - $\gamma$  Effect of phloridzin.
  - (b). Permeability to phloridzin.
3. *Summary.*

1. *Experimental evidence of the change of permeability (vital permeability) to electrolytes.*

As was hinted at in my first lecture, I first showed in 1889 that, contrary to what I had assumed in the beginning, the blood corpuscles are not semi-permeable but apparently could let electrolytes pass. Three years later, in 1892, new experiments made by me confirmed this result. These experiments were of importance also, since they, for the first time, brought out—and I will stress this point—that this permeability has no constant value, but

is subject to changes that depend upon the processes of life, and is therefore a vital permeability.

(a) Permeability of red blood corpuscles under the influence of physiological amounts of CO<sub>2</sub> (1892). Significance in physiology and pathology. Experiments of Girard.

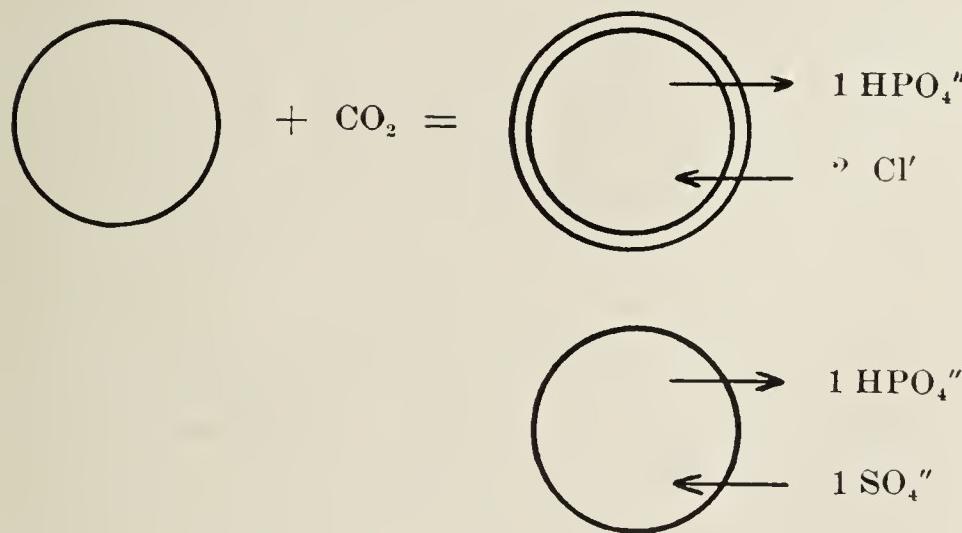
Our starting point was the following: In view of the fact that we had found the haemolysis of the red corpuscles to be so sensitive to slight modifications in the concentration of the surrounding fluid, we put to ourselves the question whether the erythrocytes of the arterial and venous blood of the same animal would show any difference in the salt concentration in which coloring matter was discharged. Such a difference indeed proved to exist: the carotid blood of a horse, for instance, showed a commencement of the loss of coloring matter in a 0.61% NaCl solution, the jugular blood, on the other hand, in a 0.62% NaCl solution.

Observations of this slight difference became the starting point for many investigations, conducted by myself and others, which are still going on. Before entering upon this subject, it may be well to impress upon young research workers who may be present in this audience how important it is not to neglect trifling differences, apparently not of great moment, such as between 0.62 and 0.61 per cent.

Now the question arose, what was the cause of this slight difference? It was obvious that the cause should be sought, to some extent at least, in the difference of CO<sub>2</sub>. If this hypothesis were correct, then blood taken from the

jugular vein and treated with  $\text{CO}_2$  would have to show, even more intensely, the same phenomena. And indeed, it appeared that after this treatment the blood corpuscles began to lose coloring matter in a 0.89 per cent  $\text{NaCl}$  solution instead of in a 0.62 per cent concentration. We now asked ourselves the question: Is there a modification of the composition of the blood corpuscles also associated with this change in concentration? Such was indeed the case. The effect of  $\text{CO}_2$  proved to be very great and led to the study of the influence of carbon dioxide on the distribution of the constituents of the blood among the corpuscles and the serum.

In the first place it appeared that when blood is treated with even a small quantity of carbon dioxide, *a quantity which is still within the range of the physiological margins*, an interchange of constituents takes place between blood corpuscles and blood serum. When the dioxide was driven out, the original partition was fully restored again; consequently, the phenomenon appeared to be reversible, and the conclusion was drawn that it must be of importance to life. For the blood, while it flows through the capillaries of the tissues, is acted upon by carbon dioxide, and when it reaches the lungs, the carbon dioxide is expelled again. What then are the changes which the carbon dioxide brings about in the distribution of the blood constituents among the blood corpuscles and environment? What happens can be represented by the following scheme:



When  $\text{CO}_2$  is acting on the blood corpuscles,  $2 \text{Cl}'$ -ions will be replaced by  $1 \text{H}_2\text{PO}_4''$ -ion. Since all ions, whether bi- or polyvalent, have the same water-attracting power, the cell will attract water and swell, as represented by the outer circle.

The lower part of the figure represents the exchange of  $\text{H}_2\text{PO}_4''$  and  $\text{SO}_4''$ . This exchange has no influence on the volume of the blood corpuscle, because both ions are bivalent.

The most important change appeared to be the passage of chlorine into the blood corpuscles and the increase of alkalinity, I mean the amount of diffusible alkali of the blood serum, and also a considerable swelling of the blood corpuscles. With every act of respiration, therefore, rhythmical changes take place. Thus, while the blood circulates through the tissues, the blood serum becomes

more alkaline, poorer in chlorine and phosphoric acid and poorer in water. Owing to the latter, the concentration of albumin and other organic substances in the plasma increases in consequence of the inspissation. When the blood reaches the lungs again, the carbon dioxide is expelled, the volume of swollen corpuscles decreases and the relation of the constituents is as before. These results could be confirmed by comparative experiments on normal carotid and jugular blood. Recently van Slyke in an extensive research was able to describe the same influence of  $\text{CO}_2$  on the corpuscular permeability to ions.

It would carry me too far if I should enter upon the significance of these phenomena to various physiological and pathological processes. As to physiological processes, I wish to draw your attention only for a few moments to *metabolism*.

When the blood, after having circulated through the arterial system, reaches the capillaries, it there receives  $\text{CO}_2$  from the tissues. As said before, this causes the blood corpuscles to take up water from the plasma, in consequence of which the percentage of nutritive material in the plasma (protein, sugar, fat) increases; and its alkalinity is also raised thereby. The advantages of this are of two kinds:

(a) Now it is possible for the plasma to offer the tissues a larger amount of nutrition than would be the case if the blood were still purely arterial. Without this regulation, the nutritive capacity of the plasma would decrease, and the more so, the larger the capillary field it had traversed. Now, it is obvious that the longer the blood circulates in the capillary net, the greater the amount of  $\text{CO}_2$  it takes up and the higher also will be the concentration of the nutritive substances in the plasma of the blood. And from this the tissues must receive their pabulum.

It may be observed, in passing, that we have here an interesting *chemical case of Weigert's law*, which also plays such an important part in wound healing and in the production of anti-bodies in curative serum. This law, we all know, says that any loss of tissue, if followed by excessive regeneration, is repaired by overcompensation. And what is the case here? Although the tissues have withdrawn numerous substances from the plasma during its course through the capillaries, the concentration of the venous plasma, in spite of this loss, is still greater than that of the arriving arterial plasma.

(b) Since not only the percentage of protein, fat and sugar, but also the alkalinity of the plasma have increased, the conditions for oxidation in the tissues have become more favorable.

That alkali furthers oxidation is indeed a well-known fact. In this connection the behavior of pyrogallic acid may be mentioned. An aqueous solution of this substance takes up relatively very little oxygen. If, however, a certain amount of alkali is added, the capacity to take

up O is considerably increased. Chemistry can add to this example many others.

And physiology also contributes arguments for this conception. Thus Curt Lehmann has shown that metabolism, i.e., the taking in of O and elimination of CO<sub>2</sub>, is raised considerably after the introduction of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) and glucose administered by mouth or intravenously, a phenomenon which does not occur if glucose alone is injected. Hydrochloric acid, however, has a retarding influence on metabolism. The work of the Nutrition Laboratory of Boston, carried on by F. G. Benedict and his co-workers, is very important in this connection. Now let us consider a few points which show the significance of CO<sub>2</sub> in *pathology*.

It is obvious that in the case of venous congestion the interchange between blood corpuscles and plasma is much more pronounced than in the normal circulation. In this connection I might say that these statements about CO<sub>2</sub> proved useful to American investigators during the war.

It may be noted, too, that these facts have also formed the basis for the well-known researches of Korányi on the diagnosis and treatment of heart diseases. This clinician has shown that decompensated heart disease can be differentiated from insufficiency of the kidney. In both cases the lowering of the freezing point of the blood serum is increased, in the case of heart disease through the accumulation of carbon dioxide, and in the case of renal insufficiency through the accumulation of waste products of metabolism which the kidney is unable to excrete. In both cases a decrease in the amount of chlorine in the blood plasma is observed. To distinguish between these two diseases, Korányi suggested that oxygen should be passed through the blood drawn from the patient. If the case is one of decompensated heart disease, there is an increase of chlorine and the lowering of the freezing point of the serum also becomes normal again. If these phenomena are absent, then the case is one of renal insufficiency.

Pursuing the same train of thought, Korányi also worked along therapeutical lines. In cases of decompensated heart disease he advises the inhalation of oxygen. With its use the surplus of carbon dioxide disappears and with it the cyanosis. The blood further undergoes the same changes which are observed *in vitro* when oxygen is passed through it; the blood corpuscles surrender water to the plasma with the result that the percentage of albumin decreases and the concentration of chlorine increases. It is obvious that, in consequence of this dilution of the plasma with water, the viscosity of the blood will diminish, and the circulation be improved. This improvement of the circulation serves a double purpose: first, the blood of the coronary system will feed the heart better than when the flow of blood is slow on account of the great viscosity of the plasma. In the second place, the functioning of the kidney is improved; the diuresis

increases and this is still further facilitated by the increased amount of chlorides in the plasma. A special value of the oxygen inhalation further lies in the fact that through the improved action of the heart and increased diuresis the œdema disappears. What is especially remarkable is that the beneficent after-effects of the oxygen-inhalation last such a long time.

The recent detailed researches of Yandall Henderson on shock, acapnia, and ether-anæsthesia, are partially based also upon my experiments on the influence of the respiratory exchange of oxygen and carbon dioxide, and on the interchange of substances between red corpuscles and their environment.

But let us return to our own experiments on the effect of carbon dioxide on permeability. These soon gave rise to the question whether the interchange of constituents was caused by the carbon dioxide as such, or as an acid. The latter proved to be the case, because other acids also, like sulfuric acid, hydrochloric acid and lactic acid, brought about the same interchange, whereas the addition of alkali to the blood caused a reverse action.

Afterwards, in 1901, it became clear to me that we have here a general principle, for not only red corpuscles but also white blood corpuscles and other isolated cells, suspended in their natural media, showed swelling in carbon dioxide and other acids, and shrinkage in traces of alkali; moreover, organs *as a whole* showed the same phenomenon. In this way a definite swelling of the kidneys was demonstrated with the plethysmograph, when a trace of acid was added to the blood, and a shrinkage when the blood was treated with a little alkali. In 1906 these experiments were repeated by the Belgian physiologist Demoer with the same result, and were also confirmed by Martin Fischer. In this connection cloudy swelling of the kidneys and other organs in the course of infectious diseases is to be thought of.

Till now stress has been laid upon the permeability of the blood corpuscles to chlorine, but the blood corpuscles appeared to be permeable also to other acid radicals, as, for instance, to SO<sub>4</sub>, NO<sub>3</sub> and phosphoric acid; these, also, it appeared, could pass through. This, however, could be demonstrated along quantitative chemical lines more clearly with blood corpuscles which had been treated with CO<sub>2</sub> than with purely arterial ones. I shall not dwell now upon the explanation of this fact. Only one remark. The objection has been made that the CO<sub>2</sub> had harmed the blood corpuscles so that they were no longer normal. This objection is not justified, however, because the amount of CO<sub>2</sub> which is added to the arterial blood does not need to be more than that which corresponds with the physiological quantity, and moreover because the process is totally reversible.

Using other methods, Dr. S. de Boer, working at my suggestion in the Groningen Physiological Laboratory, has recently demonstrated this phenomenon anew with

reference to  $\text{SO}_4$ . If blood from the carotid is shaken with 4 per cent by volume of  $\text{CO}_2$ , which is hardly the physiological amount, and of the serum an ultra-filtrate is made, it is found that this ultra-filtrate contains more  $\text{SO}_4$  than does the ultra-filtrate of the carotid blood which has not been shaken with the physiological quantity of  $\text{CO}_2$ . It was also observed that the ultra-filtrate of the jugular serum contained a little more  $\text{SO}_4$  than the carotid serum.

These  $\text{SO}_4$ -determinations were made by a micro-volumetric method devised in our laboratory by which, it may be said in passing, *free  $\text{SO}_4$  had for the first time been demonstrated and quantitatively determined in the blood.* It would take me too far to go deeper into the significance of the reversible influence of  $\text{CO}_2$  for physiological and pathological processes.

It may only be remarked that the chief significance of the  $\text{SO}_4$  exchange between corpuscles and serum lies in the promotion of the removal of  $\text{SO}_4$  from the body. For, when the blood passes through the capillary vessels, the  $\text{CO}_2$  that has been formed in the course of the oxidation will cause the  $\text{SO}_4$  in the blood plasma to pass partly into the corpuscles, and thus pave the way for the passage of a fresh quantity of  $\text{SO}_4$  from the tissues into the capillary vessels.

The corpuscles charged with  $\text{SO}_4$  pass into the lungs, and under the influence of oxygen transfer to the plasma the  $\text{SO}_4$  that has been absorbed by the capillary vessels of the tissue. The plasma, which has thus received an additional quantity of  $\text{SO}_4$ , passes through the kidneys and there releases some of the  $\text{SO}_4$ , and now in a more ample measure than would have been the case if the  $\text{SO}_4$  percentage of the plasma had been smaller.

We have, therefore, to deal with another example of an extremely adequate rhythmical influence of the respiratory exchange.

I will only still lay stress once more upon the fact that the same changes in permeability of red and white corpuscles and other cells, brought on by carbonic acid, are also caused by other acids and that, on the addition of an alkali, the opposite changes take place. To elucidate by an example what I mean: if blood be treated with  $\text{CO}_2$ , a swelling of the blood-corpuscles is seen; the same thing happens if traces of hydrochloric acid or sulphuric acid are added, whereas shrinkage of the blood cells ensues on addition of traces of alkali. These phenomena are reversible, indicating that they are *vital* processes.

Many years ago, Koeppe and myself gave an explanation for these facts, the principle of which, ionic interchange between cell body and environment, has been accepted by almost all workers in this field. I shall not dwell upon this question, but I only wish to draw attention here to the experiments of Girard with simple membranes, which are very instructive. These experiments were founded upon the theories of Terrin on electro-os-

motic, which have of late been extended in the beautiful researches of Jacques Loeb on osmosis and electrification of water.

When a solution of barium chloride, mixed with a little ammonia or a little  $\text{HNO}_3$ , is subjected to diffusion with distilled water, the relative quantities  $\text{Cl}$  and  $\text{Ba}$  which pass through the polarized membrane during the same length of time are very different as the following scheme shows:

	DIVIDING MEMBRANE	MEMBRANE
Distilled water	—   + —   + $\text{BaCl}_2 \frac{n}{50}$ + —   + $\text{HNO}_3 \frac{n}{100}$ + —   +	Distilled water +   — +   — $\text{BaCl}_2 \frac{n}{50}$ + +   — $\text{NH}_3 \frac{n}{100}$ + +   —

Here 6 gr.ion  $\text{Cl}$  against 1 gr.ion  $\text{Ba}$  diffuse into the distilled water in 20 minutes.

Here  $\frac{1}{2}$  gr.ion  $\text{Cl}$  against 1 gr.ion  $\text{Ba}$  diffuse into the distilled water in 20 minutes.

Without polarization of the membrane there had to be a diffusion of 1  $\text{Ba}$  against 2  $\text{Cl}$ . Here, however, the specific motility of the ions had not been taken into account. Hence the conclusion may be drawn that the effect of hydrogen-ion-concentration on the partition of the constituents of corpuscles and serum must be considerable.

In life the circumstances are more complicated than in the scheme of Girard. I am thinking here of the interchange in tissues between fixed cells and fluids which are in motion; e.g., here the principle of the initial rate of osmosis, studied by Lazarus Barlow, is to be taken into account.

Thus far we have considered especially the permeability to salts and their components, the electrolytes. Very likely, therefore, the question may be put by some of my audience whether this can be justified and whether the electrolytes have indeed such a great significance. My answer to this is that, in the first place, the salts or their ions by virtue of their water-attracting properties (osmotic pressure) exert an important motive force on the passage of fluid between the cell and its surroundings; secondly, they strongly influence its dispersiveness; hence the viscosity of fluids and cells. We recall here *inter alia* the influence of the motility of the cell contents in the case of cell division and fertilization.

#### (b) Influence of $\text{MgSO}_4$ on intestinal epithelium.

We now leave the subject of permeability changes brought on by  $\text{CO}_2$ . This having been established in 1892, the changeability of permeability, due to magnesium sulphate, was shown by me in 1898. At that time I was able to show by means of volumetric determinations that intestinal epithelium, after it had been in contact with a solution of  $\text{MgSO}_4$ , allowed the passage of common salt only with great difficulty. In this connection I must refer to the experiments of MacCallum.

This result is in agreement with the experiments by Hay who had shown the purgative action of  $MgSO_4$  to depend partly on a decrease in resorptive power of the intestinal mucous membrane. Hay was able to demonstrate that the animal, after having taken in  $MgSO_4$ , tolerates quantities of strychnine which otherwise would have been immediately fatal. That the influence of  $MgSO_4$  on permeability is reversible is well known to any one who ever took a dose of Epsom salts or prescribed it for his patients.

*2. Experimental evidence of the change of permeability to anelectrolytes (glucose).*

Thus far we have dealt with changes of permeability in cells with reference to inorganic substances, by which I mean electrolytes; I now ask your attention for the changes of permeability to organic substances, particularly to glucose.

(a) Permeability of glomerular epithelium to glucose.

These experiments chiefly related to the permeability of the glomerular epithelium to glucose and to the profound influence which is exercised on it by very minute physiological changes of the liquid that flows through the capillaries of the glomeruli. Allow me to make a few remarks about this and I shall try to do it in a chronological order.

I was led to undertake these researches by the following considerations.

Since the time of Claude Bernard it has been known that sugar is never absent from the blood and that it rarely appears in the urine of normal individuals. What is the cause of this and why does sugar appear in the urine when its presence in the blood exceeds a certain limit? These are questions which should be of interest not only to the physiologist, but also to the clinician. Several possibilities that would explain the absence of sugar in the normal urine had been considered. I shall not dwell here on these possibilities; I only wish to point out that the discussions about these possibilities have not reckoned with the fundamental question whether the kidney allows free glucose to pass through or not.

That free glucose really is present in the circulating blood, we know from the ingenious experiments on the living animal, conducted by Prof. Abel in 1913 and 1914. His experiment was as follows:

The blood, issuing from an artery through a cannula, is made non-coagulable by the addition of small amounts of extract of the heads of leeches, run into it from a side-tube, and is then caused to pass through a series of collodion tubes, immersed in warm Ringer solution. Collodion, like parchment paper, is impermeable to colloids. After passing these tubes, the blood is returned to a vein and thus is kept in continuous circulation through the dialyser. In its passage, it gives up the diffusible substances which it contains to the outer fluid, in so far as

they are not already present in equal concentration therein. By a sufficiently long continuation of the process, these substances pass out until they are in equal concentration in the blood and the outer liquid. By this method, to which the discoverer gave the name of *vividiffusion*, Prof. Abel demonstrated that the blood contains glucose in a free state.

Our experiments on the question whether the kidney allows free glucose to pass through were made with a perfused kidney. To ensure that the perfusing liquid should contain free sugar exclusively and not sugar as a colloidal compound, a Ringer solution containing sugar was taken. If the concentration of sugar in the so-formed artificial urine should prove to be the same as that of the original perfusing liquid, the conclusion could be drawn that the kidney is permeable to glucose. After this it could be determined whether addition of serum causes a retention of sugar. Should this be the case, it would be practically certain that there is present in the serum a substance which binds part of the sugar and converts it into a form that the glomerular epithelium does not allow to pass through, and eventually it could be investigated what the substance in the serum is. With these investigations Dr. Brinkman and I have been busied for more than two years; through them we have come to the most unexpected results, which now and then led us into side-paths, which, considered from a general point of view, were important in themselves.

Frogs were exclusively used for the experiments; the kidneys were perfused through the aorta at a low pressure with Ringer solution ( $NaCl$  0.7%,  $KCl$  0.01%,  $Na-HCO_3$  0.02% and  $CaCl_2$  0.02%) containing 0.05% of glucose. The perfusion liquid stood at a level of  $\pm 60$  cm. above the body of the frog. In this way approximately 150 c.c. of Ringer's solution flowed through the kidneys per hour.

The urine is—and this is of great importance—to be considered as a product of the glomeruli. To grasp this, a knowledge of the way the blood vessels run in the frog is necessary; in contrast to what we find in warm-blooded animals, the arteria renalis supplies the glomeruli and to a very small extent the tubuli, while the latter get their blood supply almost wholly from the vena portæ renalis which carries off the greater part of the blood from the hindquarters. Now, it is found that if a liquid is made to flow through this vessel under a pressure of 60 cm., not a drop of urine is excreted. Excretion takes place only under a much higher pressure, and even then very slowly. It is therefore clear that in our experiments, in which the liquid is made to flow through the arteria renalis, the urine can be derived only from the glomeruli.

The fact that it is possible to separate the contents of the glomeruli and the tubes so well from each other makes the frog an admirable object for the study of the

formation of the urine. British authors (Bainbridge and others) are very well acquainted with this fact.

In the beginning of our experiments the percentages of glucose in the urine and in the perfusion liquid were always found to be the same. These results were also obtained after ligation of the renal portal vein. It was therefore obvious that the urine obtained was a purely glomerular filtration so far as glucose was concerned. The conclusion was that the glomerular membrane is permeable to glucose.

a Effect of free calcium ions, also in other cases (red blood corpuscles, spasmophilia, stomach, rectum, œdema and capillary contraction).

#### Estimation of Ca-ions.

But further experiments, performed for a reason I shall not dwell upon here, showed that the usual Ringer fluid employed was not the physiological one for the perfusion of the kidneys. Systematic changes of the fluid taught us that for the permeability of the glomerular membrane the concentration of the free calcium ions is a factor of preponderant importance. The concentration of Ca-ions itself is governed not only by the concentration of the Ca-salt, but also by the concentration of the Na-HCO<sub>3</sub> present in the perfusion liquid. *By using the right concentrations of CaCl<sub>2</sub> and of NaHCO<sub>3</sub>, the urine was obtained sugar-free;* in other words, when the concentration of the free Ca-ions in the solution had a certain fixed value, the glomerular membrane showed itself impermeable to physiological quantities of glucose.

The concentration of the NaHCO<sub>3</sub> in the usual Ringer solution proved to be too low. This assertion requires an explanation. We, too, had found in the experiments in question that NaHCO<sub>3</sub> was necessary for the perfusion liquid; not a trace of glucose was retained in the absence of NaHCO<sub>3</sub>. Most investigators in their perfusion liquids use 0.02% NaHCO<sub>3</sub> and in the beginning we did the same. As said before, we found, however, that this concentration is too weak for perfusion through the frog's kidney, for if to the solution made up of NaCl 0.6%, NaHCO<sub>3</sub> 0.02%, KCl 0.01% and CaCl<sub>2</sub> 0.0075%, a little neutral red, which is known to be harmless to life, is added, then it is true that the perfusion liquid is orange-yellow, *i.e.*, alkaline, but the urine becomes red, proving that it has become acid.

What now is the case with the reaction of the normal urine of the frog? If a little urine is pressed out of the bladder of a normal animal, it shows a weak alkaline reaction with neutral red. From this it appeared that the buffer concentration of 0.02% NaHCO<sub>3</sub> was too weak to keep the reaction weakly alkaline, as is the case in the normal urine of the frog. But what is still more important, *the retentive powers of the kidneys for sugar grow considerably weaker and finally disappear altogether.*

The clinicians amongst my audience will immediately think of the relation between acidosis and glycosuria.

In pursuing our experiments, therefore, we made the concentration of NaHCO<sub>3</sub> stronger. In order to know what the concentration ought to be, we determined the alkalinity of the frog's serum by the titration-method of Snapper with neutral red paper as indicator. It appeared that this corresponded to a 0.285% NaHCO<sub>3</sub> solution. Consequently, a Ringer's solution containing 0.285% NaHCO<sub>3</sub> was prepared. But then also the concentration of CaCl<sub>2</sub> had to be raised from 0.0075% to more than double that percentage. The concentration of NaCl was lowered also because otherwise the Na-concentration would have made the osmotic pressure too high.

Consequently, the perfusion liquid now had the following composition: NaCl 0.5%, KCl 0.01%, NaHCO<sub>3</sub> 0.285%, CaCl<sub>2</sub> 0.015%. The urine was perfectly free from sugar and did not become red with neutral red. These results have been fully confirmed by Bahlmann in Utrecht (1920), by A. J. Clark in Newcastle and by Barkan, Bremser and Hahn in O. Frank's laboratory in Munich (1921) and others.\*

In resuming we may conclude that the glomerular membrane has the power of retaining free glucose and that this power is governed by the chemical composition of the perfusion liquid, which cannot be that of the usual Ringer fluid, NaCl 0.6%, CaCl<sub>2</sub> 0.0075%, KCl 0.01%, NaHCO<sub>3</sub> 0.02%, but must be NaCl 0.5%, CaCl<sub>2</sub> 0.02-0.04%, KCl 0.01%, NaHCO<sub>3</sub> 0.285%.

As to the amount of calcium chloride, it may be emphasized that the percentage of this salt allowable is much greater than 0.02%, if only the concentration of the Ca-ions be the right one. And this concentration is fixed by the following formula (Rona and Takahashi):

$$[\text{Ca}] = \frac{[\text{H}^+]}{[\text{HCO}_3^-]}, \text{ where } K, \text{ a constant, } = 350.$$

The [H<sup>+</sup>] can be kept constant in the artificial perfusion liquid by the addition of a small quantity of CO<sub>2</sub> or a little HCl and can be controlled by the addition of neutral red. The color obtained in this way must be the same as in a definite mixture of primary and secondary phosphate, as was pointed out by Sörensen. The concentration of [HCO<sub>3</sub><sup>-</sup>] depends on the amount of NaHCO<sub>3</sub> and this amount can be determined by titration of the normal serum. It is obvious that in this way an increase of the concentration of the calcium salt is immaterial to the concentration of the free [Ca], and as already stated, the latter dominates.

\* There is some controversy about the question whether it is the glomerular membrane that retains the sugar, or the epithelium of the tubuli, which owing to the suitableness of the perfusion liquid prevents reabsorption of the sugar leaving the glomerular membrane (A. N. Richards). It is only a question of a geographical nature.

The great significance of the concentration of free calcium-ions for the permeability of cells other than the epithelial cells of the glomeruli is also evident from experiments which were conducted in our laboratory on the red corpuscles of man and those of other warm-blooded animals, and further on the spasmophilic phenomena in muscles, on the influence exerted on the movements of the stomach, on the automatic movements of the rectum, and on artificial oedema and capillary constriction. Everywhere it is shown that the concentration of free Ca-ions is of prime importance, namely, in this sense, that it may vary only within narrow limits, too great or too small a percentage of Ca-ions being detrimental to physiological functions. Why a too large number of Ca-ions are equally harmful as too small a number will be touched upon in our third lecture.

It seems to me not out of place to say still a few words concerning the physico-chemical activities that take place at the cell-surface when acted upon by Ca and other ions, and then deal with the experiments themselves. All these experiments have convinced us that, as in most other processes, we have to deal with two antagonistic activities: the K- and Na-ion have a *softening* (lyotropic) action on the surface of the cell, the Ca-ion a *condensing* or *densifying* effect. Thus the property of the cell-surface *in casu*, the permeability, depends to a large extent on the outcome of these two antagonistic actions. It is evident, therefore, that, given a definite concentration of K- and Na-ions in the perfusion liquid, the permeability of the glomerular epithelium will have to be dependent on the concentration of free Ca-ions. Thus, to repeat once more: the Na- and K-concentration in the perfusion liquid being constant, permeability is dominated by the number of free Ca-ions, at least if the concentration of H-ions also remains constant. This has to be duly considered where the kidneys are concerned, for, as in our case, the perfusion liquid must first circulate through the muscles of the leg before entering the kidney. For this reason, the acid in the perfusion fluid has to be made saturated, which is brought about automatically by the presence of a sufficient quantity of the buffer  $\text{NaHCO}_3$ . In experiments on the antagonistic action between alkali-ions and Ca-ions, where such an acid-formation as exists in muscles and kidneys is not to be feared, as, for example, in blood corpuscles, the use of such a buffer is superfluous.

In *normal* serum the right concentration of the Ca-ions is kept fairly constant merely by an efficient buffer-action. This may be the reason why the therapeutic application of Ca-salts is so often of no value. However, when acidosis exists, the concentration of Ca-ions is altered (see the formula of Rona and Takahashi, in which it is clear that the concentration of Ca-ions is proportional to the concentration of H-ions).

Let us now turn to the experiments proving the great significance of Ca-ions for life-processes and commence with the *red blood corpuscles*.

When red corpuscles of man and of other warm-blooded animals are washed in a 0.7% solution of NaCl, they lose some of their coloring matter when they are again treated with a 0.7% solution of NaCl. But this can be prevented if a definite amount of calcium-ions is present in the solution of NaCl. The amount of  $\text{CaCl}_2$  needed for this purpose may be between 0.015% and 0.020%. If more or less Ca is added, haemolysis occurs. It is not necessary in this case to use a buffer; for no acids are formed here as is the case in the perfusion of the kidneys. I shall speak of an application of this in my third lecture, when discussing the use of physiological salt-solution for complement-titration reactions, to which Brookes has drawn attention. As I said, the significance of the calcium action is in general not bound up with the total amount of Ca, but depends on the concentration of the Ca-ions, which make up only 20 per cent. of the total Ca. (The remainder of the calcium in the blood amounts therefore to 80%, of which 50% is present colloid-bound as Ca-albuminate and 30% as a non-dissociated calcium hydrocarbonate.)

Experiments on *spasmophilic phenomena*, conducted by van Paassen in my laboratory, and which I will adduce as a third example, have also brought out the great importance of a definite concentration of Ca-ions. In 1906 J. Loeb had found already that if muscles are immersed in a fluid containing K-, Na-, Ca- and Mg-ions in physiological amount a rise of the concentration of Ca-ions causes a decrease of irritability, whereas excitability increases if Ca-ions are withdrawn, and it was found by MacCallum that in conditions of experimental tetany the amount of Ca in the blood is decreased. From this practitioners logically concluded that in tetany, characterized by an increased irritability of nerves and muscles, the administration of calcium was indicated and thus they prescribed it to their patients, frequently with good results. Attempts, however, to account for this favorable calcium action were not successful. Neither in the blood nor in the organs of patients that had succumbed to tetany could a deficiency of Ca be detected and the question thus arose whether the favorable action of Ca was not due to the concentration of Ca-ions alone. To solve this problem, the concentration of Ca-ions in rabbits was decreased by the injection of sodium bicarbonate. According to the formula of Rona and Takahashi, mentioned before, the concentration of Ca-ions is decreased thereby. And indeed, on closer investigation, irritability could be shown to have increased. This was quantitatively determined by stimulating the peroneal nerve in the rabbit. By testing muscular contractions before and after injection of sodium bicarbonate, a change in irritability could be dis-

covered. And it was subsequently found that any artificial lowering of the Ca-ion concentration is invariably associated with an increased irritability. Even spasmophilic phenomena could be brought about.

In addition to the glomerular epithelium and the red blood-corpuscles, just mentioned, I will put forward, as a fourth example of the significance of the Ca-ions, the influence of the *vagus* on the contractions of the *stomach* (Brinkman and van Dam).

When observing the stomach of a newly-killed frog, one often notices spontaneous local contractions or peristaltic waves in both directions. Stimulation of the *vagus* causes strong peristaltic movements, especially in the pyloric part; at the same time one can observe a frequent lengthwise contraction.

If the stomach is perfused with a 0.6% NaCl solution ( $\text{PH}=8.6$ ), we see that the spontaneous peristalsis has disappeared after 5 to 10 minutes and that the stomach has become quite limp; the mechanical irritability has completely disappeared.

This disappearance of the *vagus*-irritability is reversible. If, after half an hour's perfusion with the pure NaCl-solution, the liquid is replaced by a well-equilibrated salt-solution (NaCl 0.5%,  $\text{NaHCO}_3$  0.28%,  $\text{CaCl}_2$  6 aq. 0.040%, KCl 0.020%,  $\text{PH}=8.6$ ), spontaneous contractions are again observed after 5 minutes. So it is clear that after half an hour's perfusion with a pure NaCl-solution the harmful action is still perfectly reversible.

How did we find out which ions of the physiological solution in this respect caused the balancing effect? It soon appeared that the addition of K-ions, to which one has to assign such an important effect in heart-perfusion, has no effect of importance here. No concentration of K-ions which can cause the return of the *vagus*-irritability can be found.

I shall not dwell upon these systematic researches but will only point out that the addition of 0.015%  $\text{CaCl}_2$  to NaCl 0.6%+KCl 0.02% caused the *vagus*-irritability to return completely, but that a concentration of  $\text{CaCl}_2$  of 0.02% and more is unable to preserve or recall the *vagus*-irritability; tonic contractions of the stomach wall also disappear again completely in this case.

We must, however, stress the fact that to obtain these results, one should take special precautions. As the liquid used here does not possess to any degree a buffer-system against H-ions, fluctuations of H occur very easily. It is necessary that the PH of this perfusion-liquid should be 8.6 and remain constant during the experiment. The use of a rubber-tube—and I lay stress on this fact for the benefit of those who wish to repeat these or similar experiments—is very dangerous in this experiment, as it nearly always makes the liquid too acid.

These precautions being taken, one can always demonstrate that a concentration of 0.015%  $\text{CaCl}_2$ , 6 aq. (and

also 0.016%) is able to balance the concentration of alkali-ions; this concentration corresponds to a free Ca of about 9 milligrammes per litre.

*It is an interesting fact that exactly the same concentrations of Ca-ions proved necessary for the preservation of the impermeability of the glomerular membrane to physiological quantities of glucose.*

To prevent these changeable influences of the H-concentration, use was made of  $\text{NaHCO}_3$  as a buffer, as in the kidney experiments. Thus, when perfusing with a liquid containing NaCl 0.5%, KCl 0.02%,  $\text{CaCl}_2$ , 6 aq. 0.04%,  $\text{NaHCO}_3$  0.28%,  $\text{PH}=8.6$ , the *vagus* is irritable: there are normal peristaltic movements and no spastic contractions. The conditions are exactly like those of the unperfused stomach in physiological conditions. Here we see again the great influence of the Ca-ion concentration.

Something similar may be observed in the *rectum* (van Creveld and Brinkman), to which I have alluded before; it may serve as a *fifth* example. It is known to you that Magnus made many investigations on the movements of the gut. A piece of intestine is simply cut out and suspended in a salt solution. Many important results have been obtained by this method, yet the conditions under which the movements can be observed differ from the physiological ones. This led van Creveld and Brinkman in our laboratory to study the movements of the rectum under more nearly physiological conditions, by perfusing the circulating system of the frog's rectum with salt solution. Into the aorta of a frog, just above the superior hemorrhoidal artery, a cannula is introduced, by which salt solution is conducted. The rectum then shows definite movements which can be registered. If a salt solution of the same composition as that employed in the kidney experiments is used, it causes the organ to become impermeable to the physiological quantity of glucose, and the rectum exhibits beautiful movements. This salt solution was again NaCl 0.5%, KCl 0.02%,  $\text{NaHCO}_3$  0.285%,  $\text{CaCl}_2$ , 6 aq. 0.02%, PH between 8 and 8.6. The concentration of  $\text{CaCl}_2$  could, however, vary between 0.04 per cent and 0.016 per cent in different frogs. This margin of fluctuation became much narrower when no  $\text{NaHCO}_3$  had been added. Then the concentration of  $\text{CaCl}_2$  could not vary more than 0.01 and 0.02% in different frogs, evidently due to the absence of a buffer regulating the concentrations of Ca-ions. I say in different frogs, for in one and the same frog the margins of fluctuation must be narrower still.

As a *sixth example* I may adduce the experiments of a similar character made with regard to *artificial oedema and constriction of the small blood vessels* (Rudolf Hamburger). If the aorta of a frog is perfused with a mixture of NaCl 0.6%+ $\text{CaCl}_2$ , 6 aq. 0.006%, oedema of the leg results. Should, however, a 0.007% solution be used, there is no oedema. Furthermore, the small blood vessels,

capillaries no doubt, contract to such a degree that no more fluid circulates when  $\text{CaCl}_2$ . 6 aq. 0.008% is used. Addition of 0.01% KCl takes the vascular spasm away. These phenomena are completely reversible. We may also add that phenomena like those found in artificial œdema and capillary contractions, and attributed by Zwaardemaker to radio-activity of potassium, may be sufficiently explained by the antagonistic action of K- and Ca-ions, the concentration of which he left out of consideration.

It is, therefore, not without importance to give a method by which the concentration of the Ca-ions can be directly determined. Such a method has been found by Dr. Brinkman and Miss van Dam.

The method is based upon the following principle of the solubility product.

To a solution containing Ca are added as many  $\text{C}_2\text{O}_4$  ions (sodium oxalate) as are needed just to reach the solubility product of  $\text{CaC}_2\text{O}_4$ . The point at which so many  $\text{C}_2\text{O}_4$ -ions are added that this product is just exceeded is ascertained by the appearance of a slight milkiness due to  $\text{CaC}_2\text{O}_4$ . It does not matter whether in the mixture of salts there are present still other ions that can give a precipitate with oxalate. It is only necessary that  $\text{CaC}_2\text{O}_4$  should be the least soluble substance which can result in the solution.

The method is correct to 2-3 mgr. Ca" per L. The value of the solubility product was tested by the measurement of the electrical conductivity of the solution.

The principle of the method can likewise be applied to the determination of other ions. The only condition is the employment of a reagent that gives a salt which is very slightly soluble with the ion whose concentration has to be measured.

Thus, to repeat once more: the cause of the phenomenon has to be looked for in Ca, which has a condensing action on the capillaries, and in an antagonistic effect due to K. In a similar way one has to picture the influence of the free Ca-ions on the glomerular epithelium. A deficient concentration of the Ca-ions causes softening of the glomerular epithelium, rendering it permeable to glucose.

The effect of vagus-irritation on the stomach-movements must, as stated before, be also ascribed to a definite concentration of Ca-ions. It is not strange that this should also be the case in nervous excitation, when we come to think that on stimulating a nerve one has probably to do with the influence of the Ca-ions on the neuromuscular junction, which is formed at the place where the nerve and the muscle meet. Why the impermeability in the presence of a definite amount of the Ca-ions in the fluid should allow a permeability again, when there is a superabundance of Ca-ions in the liquid, we shall discuss in the third lecture. Here we shall only emphasize once more that this cannot be due to the Ca-salt, but that the essential factor must be the Ca-ions. I need only remind you of Howell's experiments on the different action of K and Ca on the heart and also refer to the experiments by Sabattani in 1902, which showed different Ca-salts to

affect the heart in a different manner, since they were dissociated in different degrees.

### $\beta$ Effect of glucose itself. Renal tolerance.

So far we have dealt with the influence of ions on the permeability of cells. Now let us consider a few cases in which the glomerular epithelium is influenced by organic substances and take, as a first example, glucose and next phloridzin.

It has been found that when the amount of glucose added to a suitable Ringer's solution is greater than that which is normally present in the blood plasma of the frog, *viz.* 0.07%, all the glucose is not held back. If there is dissolved in the Ringer's solution 0.1% glucose, for instance, then part of it passes through the glomerular membrane and the amount of glucose let through increases with the amount of glucose added. It is possible to add so much glucose that it all passes through the glomerular membrane. Is this phenomenon to be ascribed to damage done to the glomerular membrane by the increased amount of glucose? In any case the glomerular membrane becomes more and more permeable, in other words, shows what we call a diminished tolerance, which even becomes nil. I shall not discuss this question here. Only I wish to remark that the same phenomenon is observed with red corpuscles, which are also made more permeable by and to glucose.

### $\gamma$ Effect of phloridzin.

The second organic substance I shall discuss is phloridzin. The general pharmacology of this substance was described by Graham Lusk in "Die Ergebnisse der Physiologie" (1912). As is well known, traces of this substance may cause glycosuria, attributed to an action on the renal tubules in man. The question arose whether phloridzin also acts on the glomerular epithelium of the frog. To this end, the renal portal vein was ligated, so that the tubuli could not exert any influence and only the glomerular membrane could be held responsible. Brinkman now found that when only 0.0004 per cent phloridzin had been added to the perfusion fluid no trace of sugar was retained. Thus we may conclude that the glomerular membrane has been rendered wholly permeable to glucose by the phloridzin added. I found that *this permeability is perfectly reversible*. When, after a perfusion with Ringer-phloridzin, a Ringer solution devoid of phloridzin and sugar is passed through, and following on that a Ringer solution in which is present the physiological quantity of sugar, all the sugar is retained once more.

We have therefore before us an additional example of the alteration of the permeability of the glomerular membrane to glucose, and in this case it is not caused by an excess of glucose itself, but by a foreign substance, namely phloridzin.

(b) Permeability of red corpuscles to glucose itself; hyperglycoplasma.

Our discussions of the permeability to glucose related only to the kidney. A question which has of late years greatly attracted attention, from both the experimental and the clinical sides, is: Are the erythrocytes permeable to glucose?

We all know that it is customary to speak of hyperglycoplasma when the total amount of blood-sugar is greater than in normal cases, but when investigating the relation between hyperglycaemia and glycosuria, one is apt to forget that the important point is not the total amount of sugar in the blood as a whole, but that everything depends upon the amount of sugar present in the plasma; one must know the degree of *hyperglycoplasma*. Of late years a number of investigators have studied the distribution of sugar between corpuscles and plasma; most of them are agreed as to the occurrence of a considerable amount of sugar in the corpuscles, but only in the case of man and in that of the dog. The experiments of v. Creveld and Brinkman, recently conducted in my laboratory, have established in a direct manner that in circulating blood glucose is exclusively present in the plasma and that the passage of sugar from plasma to corpuscles takes place when the blood is defibrinated. The passage occurs as soon as the coagulation commences and for this reason is to be observed even in the plasma of hirudinated blood. If, however, the blood is collected in a tube coated with paraffin and then centrifuged immediately, it is evident, from volumetric determinations of the relative number of corpuscles and the total amount of blood sugar, that *all the sugar is present in the plasma*. To my mind this is of great importance for the quantitative determination of the amount of sugar present. From a theoretical point of view it is interesting to add to the foregoing that the change in itself which takes place on the coagulation of the plasma, surrounding the superficial layer of red corpuscles, is sufficient to alter the permeability of the red corpuscles to sugar.

The statement that sugar is present only in the plasma is not yet generally accepted (Folin and Berglund).

### 3. Summary.

Summarizing this lecture, we have seen that the permeability of the same kind of cell is changeable and that this variability is influenced by the physiological state of the cell at the time. One could call this a *vital permeability*. It is, among other things, the composition of the fluid surrounding the cells which can alter the permeability. It is now obvious, seeing that the composition of the surrounding fluid is in a great measure dependent upon the life processes in other organs, that this variation of permeability obtains a wider significance than if the permeability were constant. Investi-

gations on the variation of permeability through the influence of the medium were commenced by us 21 years ago, first, with the study of the influence of the respiratory exchange of gases on the interchange of components between red corpuscles and other cells and their environment, and later when these experiments were extended to the epithelium of the intestine. All these investigations dealt only with the passage of inorganic substances. Only lately have investigations been made in my laboratory on the vital permeability to organic substances, namely, on the permeability of the glomerular membrane to glucose. These brought to light, in the first place, the fact, which had been unknown up to that time, that the kidney (glomerular membrane) can hold back free glucose in a salt solution, provided that this salt solution has a suitable composition. It is especially important that the amount of free Ca-ions be a very definite one and that in its turn this amount must be regulated by the concentration of the  $\text{NaHCO}_3$ . If the perfusion liquid contains a too small or a too large amount of free Ca-ions, the glucose passes through the glomerular membrane. We have here a phenomenon which applies not only to the glomerular membrane, but also to the red corpuscles, and which is also illustrated by what has been observed in my laboratory in connection with the influence of the vagus nerve on the movements of the stomach and also on the automatic movement of the perfused rectum. Also in the case of spasmophilic phenomena, in tetany and in artificial oedema and in capillary constriction, we come in contact with the mighty influence of Ca-ions. There is no doubt that, as regards the significance of the concentration of the Ca-ions, we have here a general phenomenon.

These investigations on the significance of the calcium ions for permeability have led to investigations in four directions:

(1) On the tolerance of the glomerular membrane for glucose;

(2) On the occurrence of sugar in the plasma only and not in the blood corpuscles, thus on the ideal hyperglycoplasma;

(3) On the influence of phloridzin on the permeability of the glomerular epithelium of the frog;

(4) On the cause of the so remarkable and yet so efficient impermeability to free glucose. I say efficient: Is it not a remarkable fact indeed, that glucose is retained by the kidney (glomerular membrane), whereas other crystalloids like  $\text{NaCl}$ , sulfates and phosphates pass through? To what must this peculiar behavior of glucose be ascribed? This question will be treated in the third lecture.

# SPONTANEOUS CONTRACTIONS OF THE FALLOPIAN TUBE OF THE DOMESTIC PIG WITH REFERENCE TO THE OESTROUS CYCLE

By DANIEL L. SECKINGER

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The extreme variability of smooth muscle contractions in the female internal genitalia of mammals even within individual species has been noticed from time to time and has been ascribed to changes in temperature, oxygenation and the surrounding medium. That such conditions may seriously affect the contraction type, workers in this field must agree; but even when these factors are properly controlled, striking differences may still be discerned. In the light of more recent investigation these variations seem to be due rather to intrinsic phenomena within the muscle than to outside agencies. It is these changes or variations in the Fallopian tube cycle of the pig coupled with their probable significance that we wish to consider as the basis of the present study.

In the beginning it may be said that the literature bearing upon this subject is extremely limited, consisting solely of two contributions by Blair and Keye, the former working with uteri of albino rats and the latter with uteri of the domestic pig. Blair,<sup>1</sup> in his work upon the uteri of albino rats, found definite types of spontaneous contracture, depending upon the stage of the cycle. He states that the rhythm is slowest at the time of oestrus, increasing generally to a maximum late in the resting stage, and decreasing rather suddenly at pro-oestrus, and also that barely perceptible minimal contractions were often interpolated between the ordinary maximal contractions of the uterus at and near oestrus. Blair's results in a general way parallel those of Keye which followed them, but he makes no attempt at even a hypothetical explanation of the phenomena observed. Briefly stated, Keye<sup>2</sup> was able to show that during the twenty-one day cycle in the pig, definite physiological changes in the uterine musculature, accompanying and coincident with histological changes, take place within the internal genitalia. These phenomena lead to the production of two types of spontaneous contracture, one of the nature of relatively large contractions of long duration (1.5-2.5 minutes) and the other of smaller contractions of briefer duration, which are superimposed upon the former. Their probable significance in the cycle is explained as follows: "During this time the Graafian follicles are maturing and for a short time after they burst contractions of the major type predominate or are present alone. When the corpora lutea reach maturity and up to a time shortly before they regress, the minor contractions predominate or are present alone."

Keye thinks that these contractions by their variations may be the agency in transportation of ova and early embryos in the uterine.

This paper, so far as known, records the first study yet made of the contractions of the Fallopian tube with respect to the ovulation cycle. Gunn<sup>3</sup> has called attention to the rhythmical contractions of the human Fallopian tube, but undoubtedly this observation was made without reference to the chain of events that occur in the ovary and uterus during the cycle. Matsumoto and Macht<sup>4</sup> have also demonstrated the effect of ovarian extracts upon the musculature of the pig's Fallopian tube and stress the importance of increased rhythmical contractions produced by certain of these extracts.

The domestic pig was chosen as the animal most suited for our study of tubal contractions for the following reasons: first, in this animal oestrus is regularly periodic (21 days) and ovulation occurs spontaneously; secondly, Keye, working in this laboratory, was able to show cyclic changes in the uterine contractions of the pig; last, but most important, through the contribution of Corner,<sup>5</sup> it is readily possible to follow in material obtained directly from the abattoir the anatomical changes in the uterus and ovary which underlie the reproductive cycle. Briefly stated this method is as follows: The first three days following ovulation are marked by the presence of freshly ruptured follicles, during which time ova may be found in the Fallopian tubes. The fourth to seventh day is characterized by the presence of ova in the uterus. By the seventh or eighth day the ova disappear from the uterus by degeneration, and the corpora lutea, having increased in size to a diameter of 9 mm., become solid. Gross and microscopic signs of degeneration of corpora lutea appear at the end of the second week; the corpora decrease in size, increase in firmness and change from flesh to a yellowish color. At this time the granulosa-lutein cells are degenerating. The third week is characterized by the continued degeneration of the corpora lutea and the enlargement of the Graafian follicles from the resting stage to a diameter of 8 to 10 millimeters. At their rupture the old cycle is completed and a new cycle begun.

Through the co-operation of a neighboring abattoir, female internal genitalia from swine were obtained in a fresh state, and removed at once to the laboratory, where a routine procedure for this examination was adopted.

In cases of recently ruptured follicles both tube and uterus were carefully washed with physiological salt solution in order to procure the ova. In later stages where ova are no longer to be expected in tube or uterus and while the new follicles are in a state of quiescence, careful measurements were made of the diameter of the corpora lutea; in a still later stage, diameters of both corpora lutea and the follicles developing for the next ovulation were obtained, the purpose being to assign to every specimen a tentative date in the cycle. In every case pieces of the ovary and uterus were fixed in Bouin's fluid from which microscopical sections were prepared. The latter were studied with reference to the stages illustrated in the monograph of Corner in order to establish the exact date in the cycle.

The material for our observations consisted of the genitalia from twenty-seven animals so selected that each stage of the cycle was covered by a representative number of specimens. According to this plan the specimens were grouped in order and number as follows: Oestrus, 6 (ova in tubes, 1-3 days); fourth to seventh day, 3 (ova in uterus); seventh to eighth day, 1; twelfth to fourteenth day, 2; fifteenth to seventeenth day, 3; eighteenth day, 2; nineteenth day, 1; nineteenth to twenty-first day, 7. Pregnancies 2 (12th-14th day, 15th-17th day). Total 27.

The apparatus used for obtaining records was the ordinary kymographion, set to revolve once in sixty minutes, and a time clock recording one-minute intervals was attached. Fresh rings of the whole Fallopian tube, about 3 millimeters in width and taken from the middle part of the tube, were suspended in oxygenated Locke's solution at a constant temperature of  $37.5^{\circ}$ , and attached by means of a string to the short arm of a recording lever. The preparations usually showed signs of irritability from the beginning; this, however, was not invariably the case, for in several instances at least fifteen minutes elapsed before the muscle began to show signs of irritability. After a period of about thirty minutes had elapsed, the preparations, as a rule, had recovered from shock and their natural function was evidenced by the definite and characteristic contractions, which persisted thereafter. When such a stage was reached, this portion of the graph was chosen for our permanent record.

#### OBSERVATIONS

Our records show that during the oestrous cycle two definite types of contraction occur in the Fallopian tube of the pig parallel with the morphological changes known to occur in the ovary and uterus. One contraction type is associated with the period of heat, while the other occurs during the inter-oestrous period. Figure I shows the type characteristic of heat. At this stage the gross specimens contained recently ruptured follicles and in every instance ova were obtained in the washings from the tubes. From these data it can be said definitely that the specimens were of the period from 1-3 days following

ovulation. The tubal contractions for this period are characterized by their rapidity (13-15 per minute) and by their variations in amplitude, which are in most cases of the nature of definite undulations. This type of contraction, without exception, was obtained in the case of every animal examined by us during the oestrous interval. Profound changes occur, however, after the ova have passed into the uterus about the fourth day. At this stage of the cycle the corpora lutea are in the process of formation and ova were recovered from the uterine washings. The changes that occur in the ovary during the entire inter-oestrous period are as follows: The corpora lutea increase in size to a diameter of 9 millimeters and become solid at the seventh or eighth day. By the end of the second week the granulosa-lutein cells of the corpora show signs of degeneration and in the gross one sees that they have diminished in size and have changed from a flesh to a yellow color. By the latter part of the third week the developing follicles have reached a size of 8-10 millimeters in diameter, while the corpora show evidences of further regression.

At approximately the nineteenth day the contraction type of the Fallopian tube changes once more, jumping suddenly from five to ten contractions per minute, and there are indications that the undulations, so pronounced during oestrus, are reappearing (Fig. 3). At this time the new crop of follicles immediately preparatory to rupture have reached a diameter of eight millimeters. Figure 4 shows an accentuation of Figure 3. In Figure 4 the contractions are seen to have increased to 13-14 per minute, and undulations more nearly like those of oestrus are to be found throughout the whole tracing. At this period the follicles measure 8-10 millimeters and are in a condition of readiness for rupture at any moment. Cyclic changes occurring in the ovaries and uterus at this time furnish sufficient evidence for us to classify this period as late pro-oestrus, probably 19-21 days. A comparison of the contractions of this period (Fig. 4) with those of oestrus (Fig. 1) shows that there are striking similarities. Indeed it is impossible to distinguish the two, and it is thus apparent that the type of contraction characteristic of oestrus actually begins during the late pro-oestrous period.

Pregnancy has no effect upon the contraction type, at least during the first two weeks. Figure 5 illustrates 12-14 and 15-17 day pregnancies. Here the contractions are identical with those of the inter-oestrous period.

Figure 6 is a diagrammatic representation of the physiological changes described in the foregoing pages, which take place in the Fallopian tube during the ovarian cycle, in comparison with a graph from Corner's paper<sup>5</sup> which portrays the changes that occur in the ovary during the cycle.

The time at which the rapid undulating contractions begin to appear is found in the different animals of our group to be correlated with the diameter of the develop-

ing follicle. The ovaries of fifteen animals out of the entire collection showed follicles already developing beyond the resting stage. Those having a diameter of more than seven and one half millimeters without exception gave tubal contractions of the rapid undulatory type (Fig. 4), while in every instance follicles measuring less than seven and one half millimeters showed the slow and rhythmical contraction of the inter-œstrous period (Fig. 2). This casual observation was further strengthened and confirmed after resort was again made to the slaughter-house, where follicles from two animals measuring exactly seven millimeters were selected, the end in view being to determine at what size of the developing follicle the rapid undulatory contradictions, characteristic of œstrus, first make their appearance. It so happened that our original collection included eight-millimeter follicles from two animals; tubal contractions from these animals were of the rapid, undulating type (Fig. 4). The two animals having seven-millimeter follicles referred to above showed definitely tubal contractions of the inter-œstrous type (Fig. 2). Hence, we are able to affirm, so far as these investigations are concerned, that the change from the inter-œstrous to the œstrous type of contraction occurs in follicles somewhere between seven and eight millimeters in diameter.

These observations, instead of clarifying, rather broadened and complicated the original problem, for not only have we been able to show that there are two types of contraction, one characteristic of inter-œstrus and the other of œstrus, but that a definite change from the one type to the other takes place in the course of the development of the follicles. It is impossible to offer more than mere speculation regarding the reason for the occurrence of these changes of such apparent regularity. Since the ovary is generally recognized as an organ of internal secretion, our attention was drawn to a consideration of its cytological elements from the point of view of cell morphology. Such a study must necessarily embrace a consideration of the corpora lutea, the interstitial cells, and the follicles. Fortunately, at this stage of the cycle, the corpora lutea can be eliminated from consideration, for by the time the rapid, undulating contractions make their appearance in the tube, the corpora have regressed to such a stage that little remains except cell debris and connective tissue. Likewise, a consideration of the interstitial cells can be eliminated, since they are not found in the domestic pig. Hence, by the process of elimination we are able with a degree of surety to confine our investigation to a consideration of the developing follicle for evidences of cytological changes that may be seen to take place synchronously with the physiological changes known to occur in the muscle contractions. Follicles from our material in all stages of development were studied histologically, with the result that the cells of the theca interna were found to present evidences of

cytoplasmic changes coincident with the physiological changes occurring in the muscle contractions.

The rôle of the theca interna in the follicle has been the subject of much discussion, which dates back as far as 1850. It is significant, however, that most of the discussion has been based upon studies of the theca cells after the rupture of the follicle, and a number of writers ascribe to these cells an important part in the formation of the corpus luteum. Several authors, among whom are Clark,<sup>2</sup> Robinson,<sup>10</sup> and Corner,<sup>3</sup> have called attention to histological changes that occur in the theca cells of the mature follicle, without any very exact reference to the time at which these changes take place. The theca cells are described by the last author as large vacuolated cells, whose vacuoles are due, at least in part, to the presence of granules of fat-like substance; the granules are alcohol-soluble, stain from a grey to deep black in osmium tetroxide, and assume a reddish color with Herxheimer's alkaline Scharlach R,—all findings which support the assumption of their lipoid nature.

The present study has revealed the fact that the cells of the theca interna undergo these definite changes only during the last stages of the development of the follicle. Figure 7 shows the structure and arrangement of the theca interna from the resting stage until the follicle has reached a diameter of approximately seven millimeters. The cells are spindle-shaped, with scanty cytoplasm and elongated nuclei. In many instances they are joined to each other by connective-tissue fibrils and in some cases by heavy fibrous bands forming a reticular framework. By the time the follicle has reached a diameter of between seven and eight millimeters (Fig. 8), the theca cells are seen to have undergone marked hypertrophy; the nuclei become rounded and somewhat enlarged; the fibrillar elements, if present at all, are scanty and delicate in outline; the cytoplasm enlarges and vacuoles appear, and the cells assume a rounded or polyhedral outline, resembling in a marked degree epithelioid cells found in tissues like the adrenal or pituitary glands to which endocrine functions have been attributed.

The physiological change from the slow rhythmical contractions of inter-œstrus to the rapid undulating type of œstrus is coincident with the morphological changes occurring in the theca interna, both changes occurring in follicles that have reached a diameter of between seven and eight millimeters. The question naturally arises as to whether there is a definite relation between the two. Reference to the literature offers little that might aid in answering this question. That the ovary has endocrine functions is a truth generally recognized, for numerous experiments have shown that their extirpation is followed by a cessation of the cyclic changes at œstrus. Robinson<sup>10</sup> states that in the ferret the follicles at a certain period of their history appear to take on the function of providing a secretion which is responsible for the phenomena of the pro-œstrus and œstrus, for "œstrus occurs only when

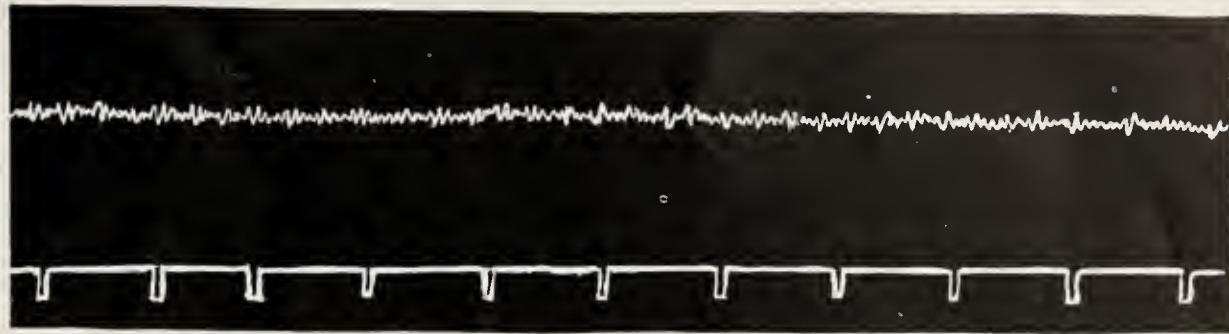


Fig. 1. Oestrus; ova in tubes. Time in minutes.

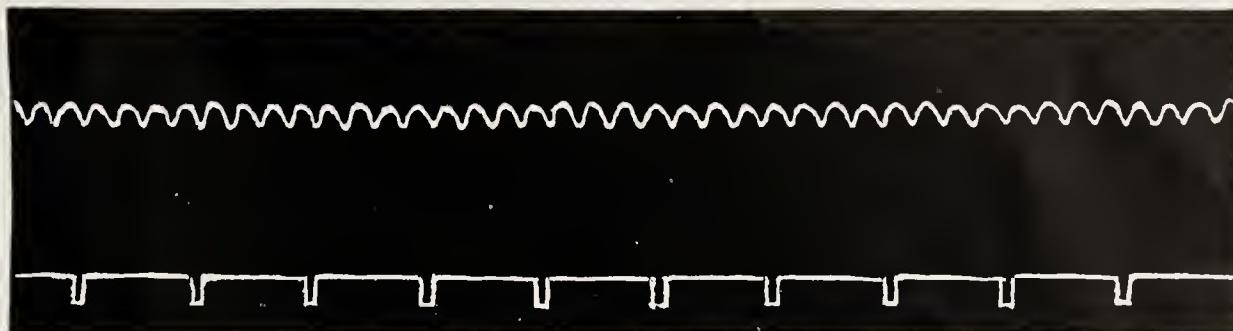


Fig. 2. Inter-oestrous period.

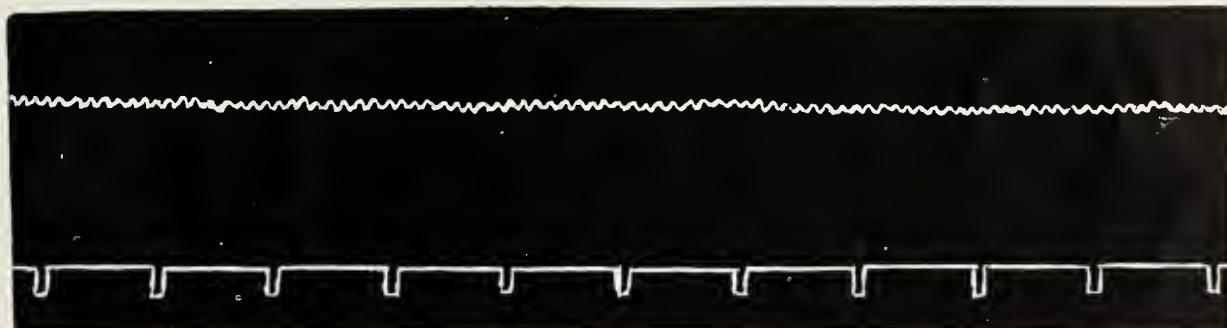


Fig. 3. Approaching oestrus; follicles 7.5 mm.

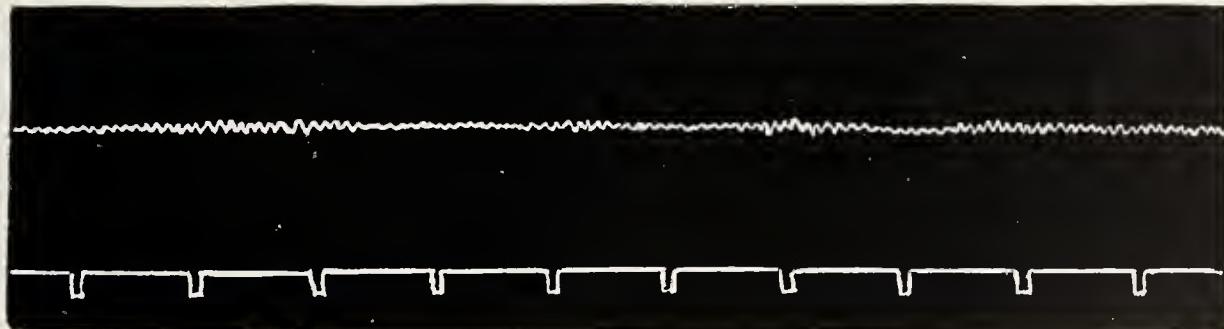


Fig. 4. Early oestrus; follicles 9 mm.

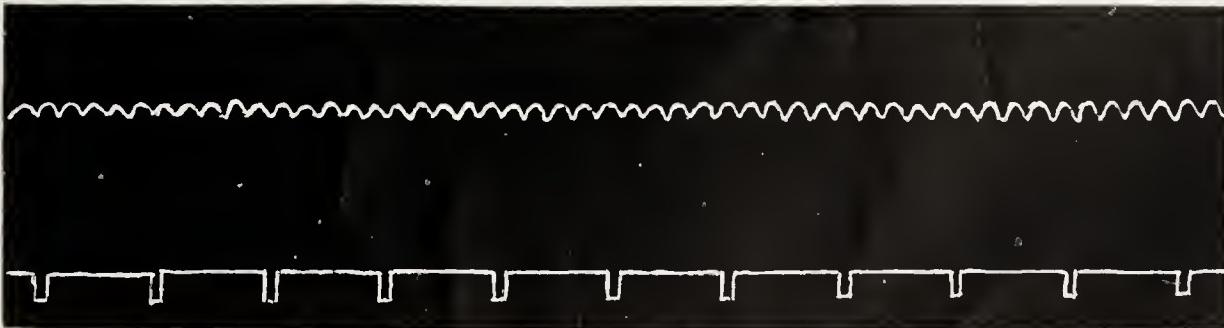


Fig. 5. Pregnancy of 2nd week.

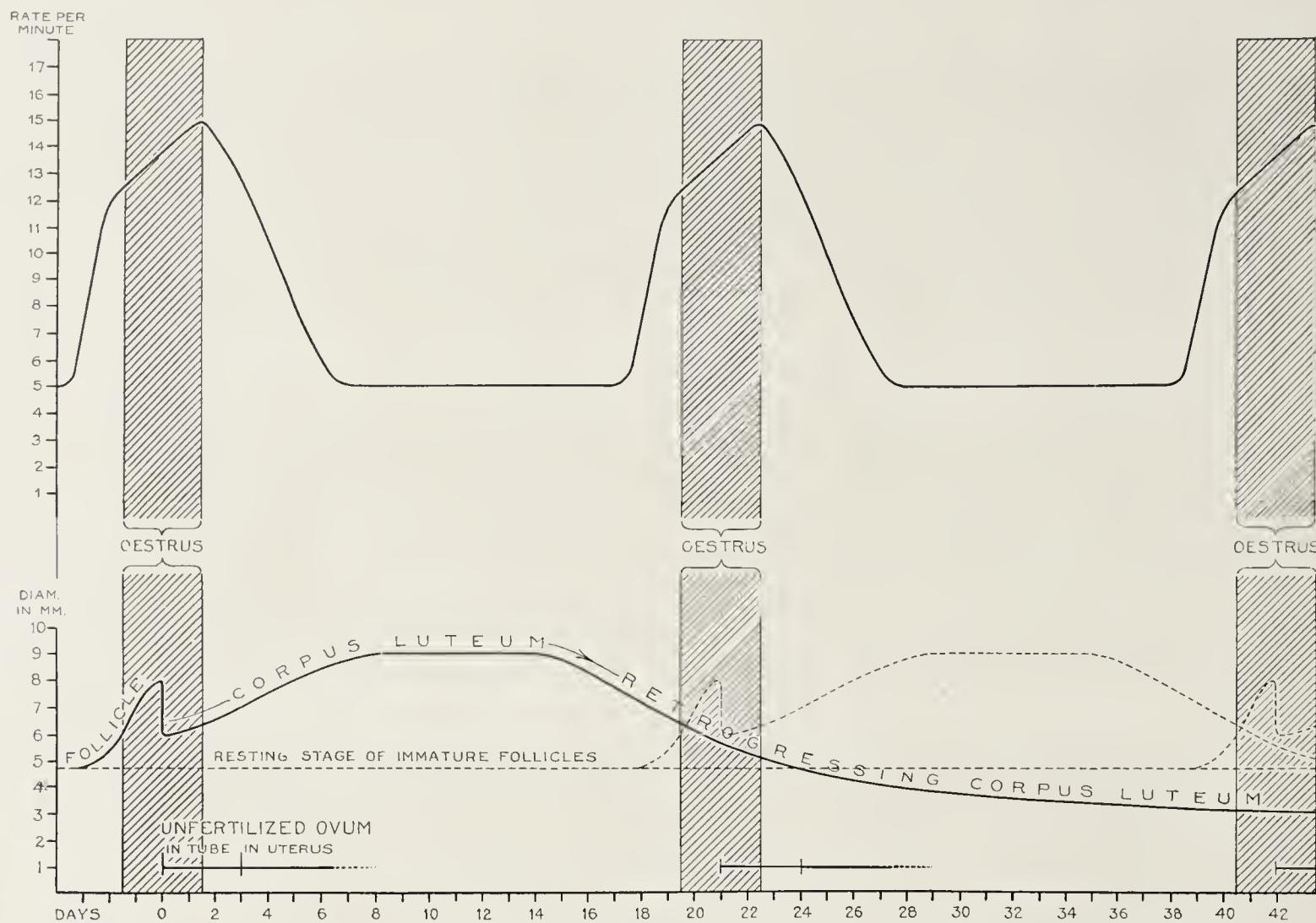


Fig. 6.—Diagram showing variations in the rate of spontaneous contractions of the Fallopian tube of the pig, in comparison with Corner's diagram of the ovarian cycle.



Fig. 7.—Photomicrograph of wall of Graafian follicle 7 mm., showing theca interna cells in the resting condition.  $\times 530$ .

Granulosa

Cells of theca interna  
in enlarged condition.

Cells of theca interna  
in resting condition.

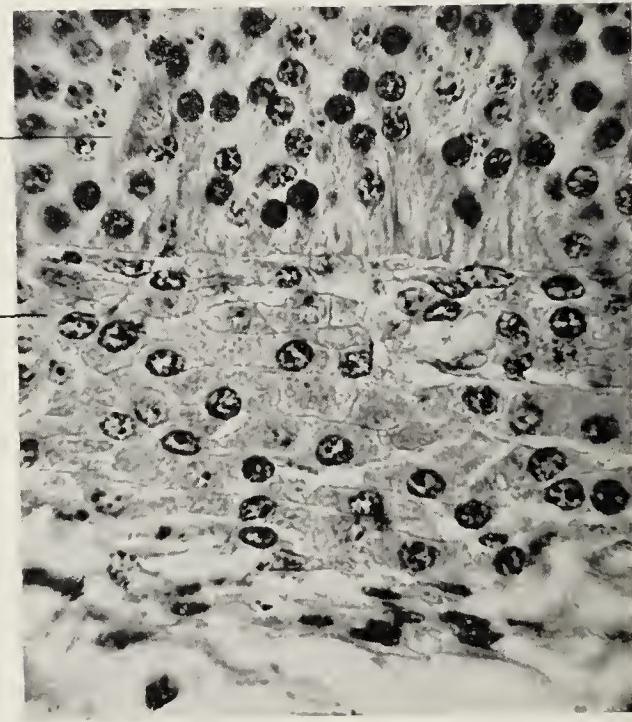


Fig. 8.—Wall of follicle 9 mm. diameter, showing theca interna cells in the mature condition.  $\times 530$ .

the ovaries contain follicles in a certain stage of development and such follicles are present as long as oestrus continues." Results obtained from the pig in this laboratory are analogous in one essential detail; the follicles do reach a certain diameter (7.8 millimeters) before the oestrous phenomena appear. But further, just at this stage, the morphological changes in the theca cells and the physiological changes in the tubal musculature offer strong presumptive evidence that there is a definite relationship between the two, and that the former by their cytoplasmic changes may produce a secretion which is responsible for the rapid undulating contraction occurring at oestrus.

Finally, the point must be emphasized that the alteration of the type of contractions occurring at oestrus presents suggestive evidence of a mechanism for transportation of the ova through the tube into the uterus. The following reasons are offered as the basis of this conclusion: First, Corner<sup>4</sup> has shown that in this species internal migration of the embryo is a fairly frequent phenomenon. On the basis of the ciliary theory of transportation, if such be true, blastocysts coming down one horn of the uterus and going up the other must of necessity move against the ciliary current; later, Corner and Snyder<sup>6</sup> showed that the uterine mucosa of the pig, except for the endometrial glands, is devoid of cilia. Moreover, Sobotta<sup>11</sup> emphasizes the fact that the Fallopian tube of the rat over its greater part is devoid of cilia, and that other factors are necessary for the transportation of the ova. Second: After the follicles have reached maturity preparatory to rupture, the rapid undulating contractions appear and continue as such uninterrupted until the ova reach the uterus. Third: This contraction type, characterized by rapid undulating contractions, seems adapted to transportation of the ova. Further analogy is found in other abdominal viscera, for it is well known that the alimentary canal is especially equipped with a smooth muscle mechanism, spontaneous contractions of which bear striking similarities to the contractions of oestrus, in response to which the bolus is carried along from point to point.

#### SUMMARY

1. Spontaneous contractions of the Fallopian tube of the domestic pig *in vitro* are characterized by two definite types which occur at regular intervals during the oestrous cycle.

2. The first type, occurring during oestrus, is marked by the presence of rapid (13-15 per minute) undulating contractions. It first appears about the nineteenth day, before rupture of the follicle, and terminates after the follicles have ruptured and the ova have entered the uterus.

3. The second type occurs during the inter-oestrous period, beginning with the appearance of the ova in the uterus (4th day), and terminates about the nineteenth day, at which time the contractions begin to revert to

the oestrous type. Contractions of this type are slow (4-6 per minute) and of equal amplitude.

4. The change from the inter-oestrous to the oestrous type of contraction of the tube takes place after the follicles have attained a diameter of 7.8 millimeters.

5. In the maturing Graafian follicles of 7.8 millimeters diameter, cytological changes in the cells of the theca interna occur synchronously with the physiological changes in the muscle contractions. These cytoplasmic variations suggest the possibility of a secretion of the theca interna responsible for the contractions occurring at oestrus.

6. Tubal contractions during early pregnancy are identical with those of inter-oestrus.

7. The oestrous type of contraction, by the nature of its definite undulations, perhaps provides a mechanism suitable for transportation of the ova through the tubes into the uterus.

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#### ERRATUM

Attention is called to the omission of the word "Cell" from the title of the article by Dr. Karl H. Martzloff, in the table of Contents and on page 184 of the June Bulletin. The title should read "Carcinoma of the Cervix Uteri. A Pathological and Clinical Study with Particular Reference to the Relative Malignancy of the Neoplastic Process as Indicated by the Predominant Type of Cancer Cell."

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